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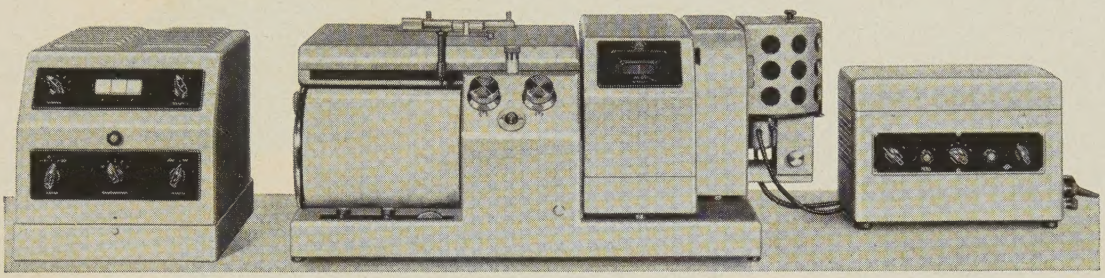

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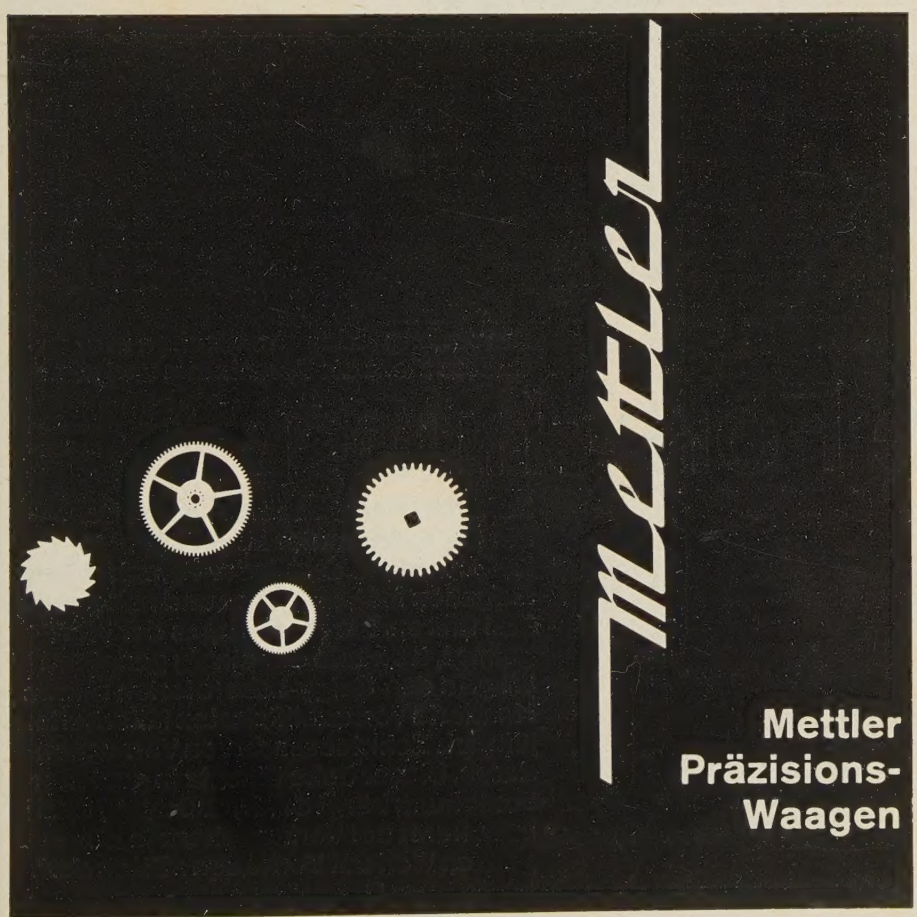

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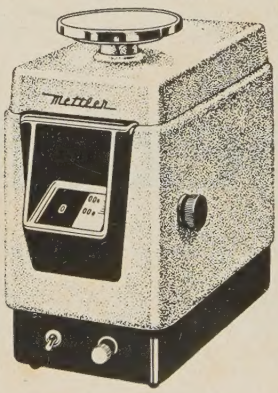

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Über Quecksilberspuren in Graphiten

Geochemische Untersuchungen an Naturgraphiten mit Hilfe einer emissionsspektrographischen Methode zum Nachweis leichtflüchtiger Elemente, der Doppelbogenmethode nach L. H. AHRENS, deren Nachweisgrenze in der vorliegenden Arbeit auf etwa 1 ppm anzusetzen ist (JANDA und SCHROLL[1]), ergaben in 104 Graphitgesteinsproben nachweisbare Quecksilberspuren [0,8–1000 g/t (ppm)]. Es war in den meisten Fällen weder eine lagerstättenmässige Abhängigkeit der Hg-Spuren noch eine Reproduzierbarkeit durch weitere Proben von ein und demselben Vorkommen gegeben. Die Konzentrationen in den Graphiten liegen in folgender Häufigkeit vor:

Gehalt < 1 ppm Hg	70 Proben	67,3%
1–9	15 Proben	14,4%
10–99	6 Proben	5,8%
100–1000	13 Proben	12,5%

Es ergibt sich um den Wert von 100 ppm ein auffallendes zweites Maximum der Häufigkeit; das eigentliche Maximum der Häufigkeitskurve liegt wohl unter der Nachweisgrenze von 1 ppm (Häufigkeit des Hg in der Lithosphäre 0,5–0,077 ppm[2,3]). Die Proben wurden teils Sammlungen von Museen und Instituten, teils auch Privatsammlungen und grubenfrischem Material entnommen. Gliedert man die Graphitproben nach ihrer Herkunft, so zeigt sich folgende Verteilung der hältigen und nicht-hältigen Proben:

Herkunft	Gesamtzahl/ Hg-hältige Proben	Hg-hältige Proben 1–9 ppm	Hg-hältige Proben 10–1000 ppm
Musealsammlung I . . .	15/11	1	10
Institutssammlung I . .	3/3	—	3
Institutssammlung II .	9/8	5	3
Institutssammlung III .	1/1	—	1
Musealsammlung II . .	4/0	—	—
Privatsammlung I . . .	47/6	4	2
Frische Probenahmen. .	25/4	4	—

[1] I. JANDA und E. SCHROLL, Microchim. Acta (im Druck).

[2] K. RANKAMA und TH. G. SAHAMA, Geochemistry (Chicago 1950).

[3] A. A. SAUKOW, *Geochemie des Quecksilbers* (Akad. Wiss. UdSSR. 1946), aus A. SAUKOW, *Geochemie* (Berlin 1953).

Die obige Tabelle zeigt, dass vor allem die hohen Hg-Gehalte Museal- und Institutssammlungen zuzuordnen sind (Musealsammlung I und Institutssammlung I, II und III), in denen gerade die Hg-reichen Graphite in den Kästen der systematischen Sammlung aufbewahrt worden sind. Der systematische Mineraloge ordnet den Graphit bei den Elementen ein, so dass meist im gleichen Kasten Stufen von Graphiten zusammen mit gediegenem Quecksilber aufbewahrt werden.

Bei frischen Proben aus dem Gelände oder Gruben ergab sich ein maximaler Hg-Gehalt von 3 g/t (ppm).

Graphit, Kaisersberg/Steiermark .	20 Proben	<0,8–3 ppm (⌀ < 1 ppm)
Anthrazit, Turrach, Kärnten	1 Probe	3 ppm
Graphit, Kropfmühle	3 Proben	<1 ppm
Kohlen, UdSSR . .	—	0,08–0,12 ppm[2]

Die Graphite, welche aus einer ausschliesslich auf Naturgraphite beschränkten Privatsammlung I stammen, weisen eine geringere Häufigkeit an Hg-Spuren auf; 50 ppm Hg enthielt eine Probe aus Batugol (Sibirien) und 1000 ppm ein Graphit aus Ticonderoga (Kanada). Es muss dahingestellt bleiben, ob diese beiden Proben nicht durch Tausch aus allgemeinen Sammlungen erworben worden sind. Nach freundlicher Auskunft von Herrn Dir. Dr. G. KLAR (Wien) trifft dies zumindestens für den Graphit von Ticonderoga sicher zu.

Die Proben aus der Musealsammlung II sind Hg-frei, da es sich um eine Lokalsammlung handelt, in der Hg-Erze nicht vertreten sind.

Es muss betont werden, dass die Proben vor der Aufarbeitung gründlich an ihrer Oberfläche gereinigt worden sind und dass, falls eine stückige Stufe zur Verfügung gestanden ist, die Oberflächenschicht (~ 1 cm) überhaupt verworfen worden ist. Trotz der zweiten Massnahme war das Hg, wenn auch in geringer Konzentration, noch nachweisbar.

Ein einfacher Versuch zeigt, dass Graphit und Kohle dampfförmiges Quecksilber absorbieren. Je eine Probe (1 g) grob gepulverten Graphites von Kaisersberg und eine Steinkohle von Grünbach (N. Ö.) wurde in einem Exsikkator 6 Wochen bei 25° ± 3°C den Dämpfen von metallischem Quecksilber ausgesetzt. In einer nachfolgenden Analyse wurde festgestellt, dass beide Proben etwa je 500–1000 ppm Hg enthielten. Der Graphit etwas mehr. Der Dampfdruck des Hg reicht aus, um bereits bei 0°C

2,3 μg Hg und bei 20°C 14,2 μg Hg/l Luft zu ermöglichen. Die vorliegende kritische Untersuchung zeigt, mit welcher Vorsicht die Analysenergebnisse von mineralogischen Sammlungsmaterial bei geochemischen Spurenuntersuchungen bisweilen zu werten sind. Dies trifft besonders für Elemente und Verbindungen mit niederem Siedepunkt, wie zum Beispiel für Quecksilber, zu.

Der Untersuchungsbefund zeigt aber auch, was dem Lagerstättengeologen schon bekannt ist, dass Quecksilbererze (auch gediegen Quecksilber) oft bevorzugt von bituminösen oder graphitführenden Sedimenten angereichert werden können. In der Hauptsache kommt wohl Zinnober (HgS) aus einem Natrium-Quecksilber-Sulfidkomplex in hydrothermalen Lösungen zur Ausscheidung. Es kann jedoch auch metallisches Quecksilber, vor allem nach Reduktionsprozessen, in der primären Aureole von Quecksilberlagerstätten als Dampf auftreten und eine Erhöhung der Hg-Konzentration über den Durchschnitt im Nebengestein bewirken. Vergleiche auch Saukow[3].

Eine Arbeit über die Geochemie des Quecksilbers, insbesondere in sedimentären Gesteinen, ist in Vorbereitung. Eine neue emissionsspektrographische Methode gestattet den Nachweis dieses Metalles bis in den Bereich seines Häufigkeitswertes in der Erdkruste.

Für die Bereitstellung von Proben danken wir den Vorständen der Mineralogischen Institute, Herrn Prof. Dr. F. Machatschki (Universität Wien), Herrn Prof. Dr. Wieseneder (Universität Wien), Herrn Prof. Dr. H. Heritsch (Universität Graz), dem Kustos des Johanneums in Graz Dr. E. Krajicek, dem Krahuletzmuseum in Eggenburg und nicht zuletzt Herrn Dir. Dr. G. Klar (Graphitbergbau Kaisersberg-Wien).

I. Janda und E. Schroll

Bundesversuchs- und Forschungsanstalt Arsenal und Mineralogisches Institut der Universität Wien, 1. Dezember 1958.

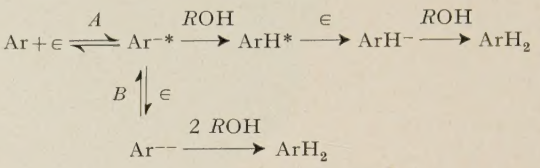
Summary

An investigation of 104 natural graphites shows in most of the samples a content < 1 ppm mercury (Hg). An emissionspectrochemical method was used by the double-arc from L. H. Ahrens. There are samples in the mineralogical collections with contents > 10 ppm Hg, if metallic mercury is present in these collections.

Reduction of Monobenzenoid Compounds by Metal-Ammonia-Alcohol Systems

The mechanism of the reduction of monobenzenoid compounds to dihydro-derivatives is of more than academic interest, since the experimental conditions in any given case must be carefully and logically chosen. Experimentally, it is found that addition of hydrogen atoms to the ring occurs: (a) in $\alpha\delta$-positions; (b) in such a way as to avoid positions substituted by dimethylamino-, alkoxy-, and alkyl groups in that order but 1,4- in a ring carrying a carboxyl group.

The mechanism was first discussed[1] in terms of possible addition of hydrogen atoms, but this idea was abandoned[2] in favour of a mechanism involving the preliminary addition of two electrons to the aromatic nucleus, followed by two protons. This mechanism shows no very obvious requirement for a proton source, and was later e. g.[3] replaced by a process involving the initial addition of one electron. The equilibria and reactions concerned can be represented by

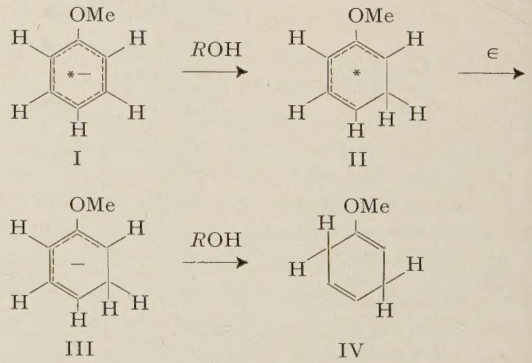


The equilibrium A is thought to be far to the left in monobenzenoid compounds and equilibrium B not present at all except in polycyclic compounds. Reduction is therefore permitted only by the presence of an alcohol, which, unlike ammonia, is sufficiently acidic to protonate the anion-radical Ar^{-}*. Subsequent stages to ArH_2 will then be rapid and probably irreversible.

Recently[4] a mechanism has been proposed which is more or less equivalent to the initial addition of a hydrogen atom. It proposes a ternary interaction of the substrate and electron and a solvated proton. None of the evidence cited is inconsistent with our mechanism. Some of it we believe to be inapplicable or incorrect. For example the alleged non-reducibility of dibenzothiophene and of isoquinoline is incorrect. These substances are reducible in one stage as well as in two experimental stages of metal addition followed by alcohol addition and there is no need to postulate a different mechanism for the one-stage process. The subsequent stages of reduction will be the same on either mechanism.

On the basis of protonation of an anion-radical, e. g. (I), we would expect the first proton to add to the point of greatest *free* charge density, i. e. *meta* to an electron-donating substituent to give (II). Further addition of an electron will then give the anion (III) which has already been shown experimentally[5] to add the second proton by a kinetically-controlled process to give (IV) which is the observed product of reduction of anisole.

This argument is simply extended to benzenes doubly substituted with electron-releasing groups in 1,3- or 1,4-positions. A particularly interesting case is (V) which experimentally is reducible to give (VI). Here initial addition should be in the unoccupied position *meta* to the methoxyl-group; $\alpha\delta$-addition would then necessarily give the observed product. In the reduction of 2-alkylanisoles both *meta* positions are unoccupied, but in practice the predominant isomer is that produced by initial proton addition *ortho* rather than *para* to the alkyl substituent to give mainly the 3,6-dihydro-derivative



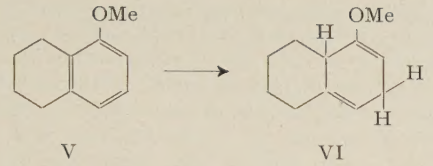
[1] A. J. Birch, J. chem. Soc., *1944*, 430.

[2] A. J. Birch (Miss), A. R. Murray, and H. Smith, J. chem. Soc. *1951*, 1945.

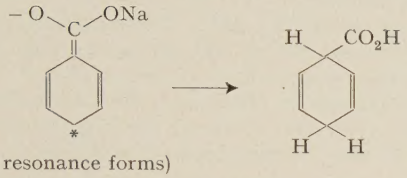
[3] A. J. Birch, J. R. Inst. Chem. *100*, 86 (1957).

[4] W. Hückel, Liebigs Ann. Chem. *614*, 417 (1958).

[5] A. J. Birch, J. chem. Soc. *1950*, 1551.



V VI



(and resonance forms)

(VII) (VIII)

The case of the electron-attracting carboxyl group (as its salt) is also clear; it should, and does, facilitate reduction by stabilising the anion-radical (VII) and also leads to 1,4-reduction (VIII).

These mechanisms are capable of explaining the effects of substituents on ease of reduction: the greater the electron-donating effects of these groups the more difficult the reduction. They explain the role of the dipolar and associated ammonia as a solvent, in stabilising the intermediate ions and ion-radicals by solvation, and they explain the necessity for a proton source and the nature of the products as noted above. A more detailed consideration of the experimental background will be published elsewhere.

A. J. Birch, D. Nasipuri, and H. Smith

Department of Chemistry, University of Manchester, December 10, 1958.

Zusammenfassung

Die Reduktion der Monobenzenoidverbindungen mit Alkalimetall und Alkoholen im flüssigen Ammoniak wird unter der Annahme diskutiert, dass die erste Stufe des Reaktionsmechanismus die Addition eines Elektrons darstellt. Dieser Mechanismus erklärt insbesondere die Orientierung der 1,4-Addition der Wasserstoffatome.

Synthese des Flavopereirins[1]

Wir haben das Indolalkaloid Flavopereirin (VII)[2], das inzwischen auch von Le Hir, Janot und van Stolk[3] sowie von Prasad und Swan[4] auf anderen Wegen aufgebaut werden konnte, folgendermassen synthetisiert (siehe Formel).

Durch Alkylierung des aus β-Äthylpyridin und Phenacylbromid erhältlichen Pyridiniumsalzes II mit dem quartären Salz des Gramins I erhielten wir III, das durch Erwärmen mit methanolischer Kalilauge zu IV (Smp. des Jodids 164°C) und Benzoesäure hydrolytisch gespalten wurde[5]. Dieses Pyridiniumsalz liess sich nach der Methode von Schöpf *et al.*[6] durch Hydrierung in methanolisch-alkalischer Lösung mit Raney-Nickel bis zur Aufnahme von 2 Mol. Wasserstoff in das nicht in Substanz isolierte Tetrahydropyridin-Derivat V überführen. Beim 16stündigen Aufbewahren von V in salzsaurer Lösung erreicht man eine Verknüpfung der beiden α-Stellungen von Indol- und Pyridinkern zum Indolochinolizidin VI [Smp. des Pikrats 211°C (Zers.)[7]]. Die (ölige[8]) Base VI lieferte bei der Dehydrierung mit Palladium Flavopereirin (Smp. des Perchlorats 329°C, Lit.[4]: 330–331°C), das mit dem natürlichen Alkaloid im IR.-Spektrum übereinstimmte.

[1] Beiträge zur Chemie des Indols, XII. Mitt.; XI. Mitt.: J. Thesing und F. H. Funk, Chem. Ber. *91*, 1546 (1958).

[2] Isolierung und Konstitutionsermittlung: O. Bejar, R. Goutarel, M. M. Janot und A. Le Hir, C. R. Acad. Sci., Paris *244*, 2066 (1957).

[3] A. Le Hir, M. M. Janot und D. van Stolk, Bull. Soc. chim. France *1958*, 551.

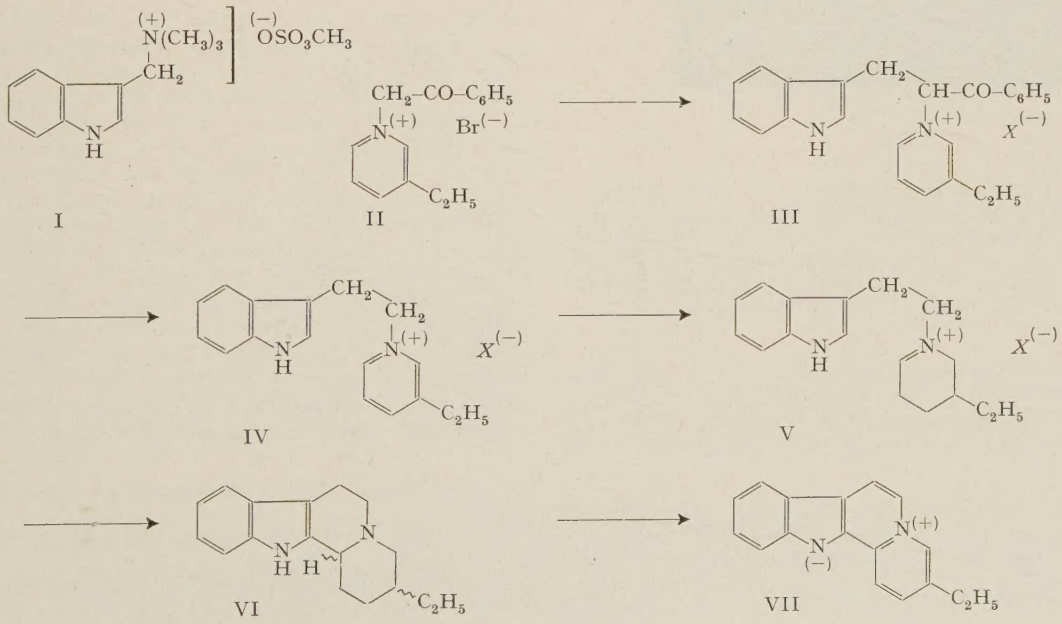
[4] K. B. Prasad und G. A. Swan, J. chem. Soc. London *1958*, 2024.

[5] Analoge Synthesen vgl. J. Thesing, H. Ramloch und C. H. Willersinn, Chem. Ber. *89*, 2896 (1956).

[6] C. Schöpf, G. Herbst, R. Rausch und G. Schröder, Angew. Chem. *69*, 391 (1957).

[7] Analoge Synthese vgl. J. Thesing, H. Ramloch, C. H. Willersinn und F. Funk, Angew. Chem. *68*, 387 (1956).

[8] N. A. Hughes und H. Rapoport, J. Amer. chem. Soc. *80*, 1604 (1958), beschreiben eine durch Hydrierung von VII erhaltene Base der Konstitution VI vom Smp. 233–235°C. Die Substanz ist vielleicht ein Diastereomeres unserer Base.



I II III

IV V

VI VII

Wir danken dem Fonds der Chemie für ein Stipendium, das dem einen von uns (W. F.) die Mitwirkung an dieser Arbeit ermöglichte.

J. Thesing* und W. Festag

Institut für Organische Chemie der Technischen Hochschule Darmstadt, 11. Dezember 1958.

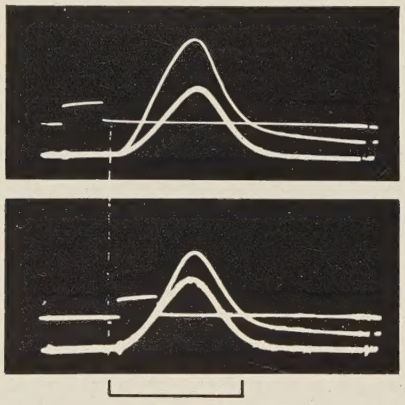
Summary

A new synthesis of flavopereirine is described, starting from the quaternary salts of gramine and N-phenacyl-3-ethyl-pyridinium bromide.

* Anschrift: E. Merck A.G., Darmstadt, Chem. Hauptlaboratorium.

Effect of Increasing the Calcium Concentration During a Single Heart-Beat[1]

Ca ions serve as a link between electrical and mechanical activity[2–4]; their concentration is an important factor determining the contractile strength. The question arises whether it is the Ca level at the *onset* of electrical activity which sets the degree of shortening (trigger effect), or whether Ca ions are essential throughout the phase of electrical activity in order to link shortening to membrane depolarization. The plan of the experiment was to raise the Ca concentration rapidly, after the beginning of an action potential, and to see whether this would still result in a stronger contraction.



10 seconds

Effect of admixing Ca ions to the perfusate of a turtle ventricle. In each of the two records, the film was exposed during three successive contractions, of which the first two coincide. Calcium was admixed, as indicated by the 2·5 sec square pulse, preceding the third beat (top record) and during the third beat (bottom record). The retouched vertical line marks the moment of stimulation.

Turtle ventricles were immersed in a bath of cooled Ringer's solution (8°C) and driven electrically at a rate of 1 beat/min. A cannula was inserted into the single coronary artery and the vascular bed was perfused with Ringer's solution of a low Ca concentration (0·7 *mM*). A calcium-rich solution (100 *mM* $CaCl_2$ in Ringer's) was kept ready in a thin tube ending inside the tip of the arterial cannula. Relatively small volumes of this solution could be admixed for periods of 2·5 sec. Contractions were recorded by means of a mechano-electrical transducer (RCA 5734). The method has been described in more detail in connexion with a different type of experiment[5].

The Figure shows the effect of momentarily raising the Ca concentration before and during systole. The lower record makes it clear that an increment of contractile strength could still be obtained if the Ca concentration in the perfusate started to rise at the beginning of systole, i.e. after the onset of membrane depolarization.

Membrane potentials were recorded on several occasions by means of Ling-Gerard[6] electrodes. A 'pulse' of calcium ions had the effect of slightly shortening the action potential. This would not, *per se*, be expected to result in a stronger contraction. It seems permissible, then, to conclude that Ca ions mediate between electrical and mechanical activity *throughout the period of membrane depolarization.*

S. Weidmann

Department of Physiology, University of Berne (Switzerland), January 23, 1959.

Zusammenfassung

An Schildkrötenherzen verstärkt eine Zugabe von Ca-Ionen, die *nach* Beginn des Aktionspotentials erfolgt, die bereits begonnene Kontraktion. Es wird daraus geschlossen, dass Ca-Ionen während der *ganzen* Erregungsphase (und nicht nur zu deren Beginn) zwischen der elektrischen und der mechanischen Aktivität vermitteln.

[1] Aided by the Swiss National Science Foundation, Grant No. 1138.

[2] I. de B. Daly and A. J. Clark, J. Physiol. *54*, 367 (1921).

[3] H.-C. Lüttgau and R. Niedergerke, J. Physiol. *143*, 486 (1958).

[4] R. Niedergerke, Exper. *15*, 128 (1959).

[5] S. Weidmann, J. Physiol. *132*, 157 (1956).

[6] G. Ling and R. W. Gerard, J. cell. comp. Physiol. *34*, 383 (1949).

Calcium and the Activation of Contraction

It has recently been proposed[1] that the contraction of heart muscle is initiated by a negatively charged Ca-compound (an 'activator') which is moved during depolarization of the cell membrane from the surface into some deeper cellular compartment. This hypothesis is based for the most part on an analysis of two experimental observations on the frog's heart: (1) that tension developed in twitches and contractures depends on the ratio $[Ca]/[Na]^2$ (these being the Ca and Na concentrations in the outside fluid)[2,3]. This observation suggested in turn that Na and Ca ions compete for a site or molecule 'R' which becomes active only when it combines with Ca. (2) Contracture tension obtained at a given ratio $[Ca]/[Na]^2$, i.e. at a given concentration of the proposed 'activator CaR', is larger the greater the degree of membrane depolarization (which was varied experimentally by altering the external K-concentration)[1]. This effect suggests that the normal mem-

[1] H. C. Lüttgau and R. Niedergerke, J. Physiol. *143*, 486 (1958).

[2] W. Wilbrandt and H. Koller, Helv. physiol. Acta *6*, 208 (1948).

[3] R. Niedergerke and H. C. Lüttgau, Nature, Lond. *179*, 1066 (1957).

brane potential acts as a barrier to the entry of 'activator' molecules, and therefore that CaR carries a negative charge.

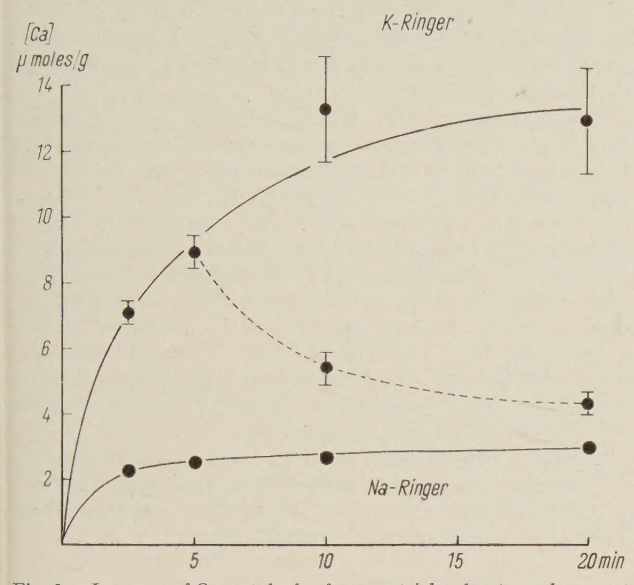
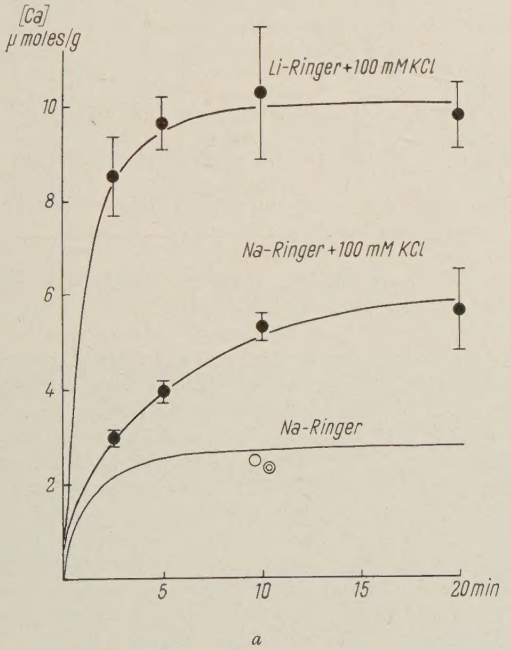


Fig. 1. – Increase of Ca-uptake by frog ventricles due to replacement of the Na in the outside fluid by K. Solid curves: amount of labelled Ca (in μ moles/g dry wt.*) taken up from fluids having either a normal NaCl concentration (Na-Ringer) or NaCl replaced by KCl (K-Ringer). Both fluids contained 1 *mM* labelled Ca. Dashed curve: ventricles exposed to Na-Ringer after a 5 min period in K-Ringer. The labelled Ca (1 *mM*) in the two fluids had identical specific activity. Each point of the upper curves is the mean of determinations with 5 different ventricles. Vertical bars: 2 × s.e. about the mean. (Ca-uptake with Na-Ringer is based on many more determinations than that with any other fluid, and its error is therefore correspondingly smaller.)

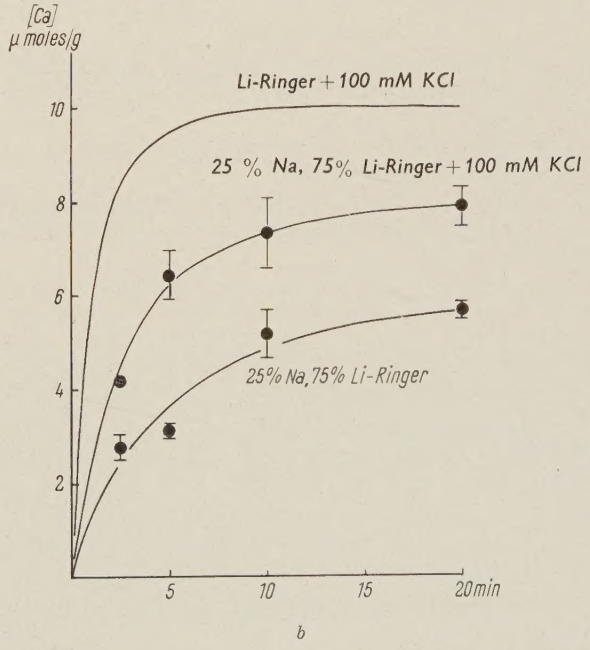
* Tissue was dried to constant weight at 80 °C.

Independent support for the existence of such a mechanism has been obtained by studying the uptake of ^{45}Ca by heart ventricles at rest and during contracture. Two series of experiments relating, respectively, to the two above-mentioned observations have been made. In the first, competition between Na and Ca was tested by perfusing ventricles with Na-depleted fluids. On the above hypothesis this should result in an increased cellular concentration of CaR and thus lead to an increase in ^{45}Ca-uptake. That Ca is taken up under such conditions has already been inferred from experiments in which it was found that heart strips accumulated ^{89}Sr when immersed in fluids in which NaCl had been replaced by either sucrose, choline Cl or LiCl[4]. This result has now been confirmed in more quantitative experiments using whole heart ventricles perfused with fluids containing ^{45}Ca. Furthermore, it has been found that Ca is taken up, to about the same extent, when the external Na was replaced by K. In Figure 1 the difference between the two solid lines illustrates this additional uptake of labelled Ca due to the total replacement of external Na by K. To find out how firmly the accumulated Ca was bound, some ventricles (dashed line) were transferred, after 5 min perfusion with KCl Ringer, into normal Ringer; both fluids contained labelled Ca of the same specific activity. The observation that ^{45}Ca is lost from the tissue under these conditions, despite a constant external concentration of labelled Ca, indicates that the cellular Ca concentration was raised during the period of Na deficiency. This supports the concept of a reversible competition between Na and Ca for a cellular site as invoked by the initial hypothesis. The additional Ca-uptake was associated with a strong contracture, while relaxation occurred early during the Ca-loss.

[4] R. Niedergerke and E. J. Harris, Nature, Lond. *179*, 1068 (1957).



a



b

Fig. 2*a* and *b*. Effect of depolarization on the Ca-uptake of ventricles. The difference between lower two curves in each illustration shows additional Ca-uptake due 100 *mM* KCl either with Na-Ringer (Fig. 2*a*) or with 25% Na, 75% Li-Ringer (Fig. 2*b*) as perfusion fluid. Upper curve in each illustration shows maximal Ca-uptake in the absence of Na (NaCl replaced by LiCl) but with additional 100 *mM* KCl. Also plotted are two controls showing the Ca-uptake in hypertonic solutions (Fig. 2*a*): in each case a 10 min perfusion period was used with Na-Ringer containing either added 180 *mM* sucrose (hollow circle), or added 100 *mM* LiCl (double circle).

The aim of the second experiment was to test whether depolarization facilitates the entry into the cell of the postulated Ca compound; such evidence would be obtained if, at a constant value of external [Ca]/[Na][2], depolarization were also to give rise to an uptake of labelled Ca. Ventricles were depolarized by raising the external K concentration to 100 *mM*; but this was done, in contrast to the first experiment, by adding KCl (solid) to the perfusion fluid. In this way the ratio [Ca]/[Na][2] was kept constant. Figure 2 shows the effect of adding 100 *mM* KCl to (1) normal Ringer's fluid (Fig. 2*a*) and (2) to a fluid in which 75% NaCl had been replaced by LiCl (Fig. 2*b*), both fluids containing 1 *mM* labelled Ca. The two lower curves, in each figure, indicate that depolarization by added K does, in fact, increase the Ca-uptake. In the absence of Na (Li-Ringer + 100 *mM* KCl, see upper curves in both figures), a maximal Ca-uptake occurs which is comparable in magnitude to that obtained in Na-free Ringer without additional K, again in agreement with the initial hypothesis. Controls in which either 100 *mM* LiCl or 180 mM sucrose were added to Ringer's fluid (Fig. 2*a*) show that neither the increased ionic concentration nor the hypertonicity of the fluids are responsible for, or contribute to, the effects shown in Figure 2.

If, as these experiments suggest, inward movement of Ca activates the contraction of the heart, an increased Ca-uptake should also occur in the course of a single twitch. Evidence for this may be found in the fact that periods of electrical stimulation also increase uptake of labelled Ca (Niedergerke, unpublished observation; cf. for similar effects in nerve[5] and skeletal muscle[6]). In this context Weidmann's recent observation[7] is of interest, viz. that the twitch tension of the heart can be enhanced by applying Ca momentarily during the course of a single action potential, which is also explicable by the initial hypothesis.

R. Niedergerke

Department of Biophysics, University College, London, January 12, 1959.

Zusammenfassung

Froschherzventrikel nehmen eine zusätzliche Menge Calcium (bestimmt mit dem Isotop ^{45}Ca) aus der Perfusionsflüssigkeit auf:

1. wenn diese Na-frei ist (zum Beispiel, wenn NaCl ersetzt ist durch LiCl oder KCl);

2. wenn dieser zusätzliches KCl zugegeben und damit die erregbare Membran bei konstanter Na-Konzentration depolarisiert wird.

Gleichzeitig mit der erhöhten Ca-Aufnahme wird in beiden Fällen eine Kontraktur beobachtet. Das Ergebnis unterstützt die früher vorgeschlagene Hypothese, nach welcher die Kontraktion des Herzens durch eine spezifische Ca-Verbindung (einen «Aktivator») eingeleitet wird.

[5] A. L. Hodgkin and R. D. Keynes, J. Physiol. *138*, 253 (1957).
[6] C. P. Bianchi, in Shanes, Pharmacol. Reviews *10*, 252 (1958).
[7] S. Weidmann, Exper. *15*, 128 (1959).

Fibrinolyse im Kammerwasser menschlicher und tierischer Augen

Fibrinolytische Fermente sind in verschiedenen Körperflüssigkeiten und Organen nachgewiesen worden[1]. Meist handelt es sich dabei um Profermente, die erst durch Kinasen, Aktivatoren oder durch bestimmte chemische Behandlung in die aktive Form übergeführt werden. Um so überraschender war für uns die Feststellung, dass im Kammerwasser menschlicher und verschiedener tierischer Augen offenbar normalerweise eine geringe Spontanfibrinolyse nachweisbar ist.

Experimentelles. Die Kammerwasser wurden durch Punktion der vorderen Augenkammer in Lokalanästhesie beim Menschen, in Pentobarbitalnarkose bei Kaninchen und Katzen und unmittelbar nach dem Tode bei Pferden, Rindern und Schweinen gewonnen und bis zur Untersuchung eingefroren. Die fibrinolytische Aktivität wurde nach der von Dausset *et al.*[2] beschriebenen, indirekten, aber sehr empfindlichen Methode der passiven Hämagglutination nachgewiesen. Das Prinzip der Methode beruht darauf, dass beim Fibrinabbau durch Plasmin eine Substanz entsteht, die tannierte und mit Fibrinogen behandelte Erythrozyten agglutiniert. Je stärker die Fibrinolyse, um so höher der Agglutinationstiter.

In Fibrinolyseansätzen ohne Streptokinase (SK) wurde die aktuelle, in Ansätzen mit SK die durch diese Aktivierungsart maximal erreichbare Fibrinolyse bestimmt. Die Ansätze enthielten folgende Komponenten:

0,1 ml Kammerwasser;
0,2 ml Rinder-Citratplasma als Fibrinogenquelle;
0,2 ml physiolog. NaCl oder 200 E SK (Varidase);
0,1 ml (4 E) Thrombin (Topostasin Roche).
Inkubation während 30 min bei 37°C.

Anschliessend wurde vom eventuell noch vorhandenen Gerinnsel abzentrifugiert und zur Agglutination genau nach der Vorschrift von Dausset *et al.*[2] verfahren.

Ergebnisse. Die Agglutinationstiter, die ein Mass für die Fibrinolysestärke sind, finden sich in folgender Tabelle:

Spezies	*n*	Agglutinationstiter	
		Ohne SK	Mit SK
Mensch .	8	1:4 bis 1:10	1:4000 bis 1:8000
Katze . .	5	1:10 bis 1:20	1:100 bis 1:400
Kaninchen	5	1:2 bis 1:8	1:40 bis 1:200
Schwein .	2	1:1 bis 1:2	1:10
Pferd . .	3	1:10 bis 1:20	1:10 bis 1:40
Rind . .	3	1:2 bis 1:10	1:2 bis 1:10

Wir dürfen annehmen, dass die durchwegs niedrigen Agglutinationstiter in den SK-freien Ansätzen tatsächlich einer geringen Spontanfibrinolyse entsprechen, da nicht geronnene, entweder fibrinogen- oder thrombinfreie Kontrollen negativ blieben. Analoge Ansätze mit Plasma anstelle des Kammerwassers sind höchstens unverdünnt schwach positiv. In der Tabelle sind die Tierarten in der Reihenfolge der SK-Aktivierbarkeit des fibrinolytischen Systems ihres Kammerwassers bzw. dessen Proaktivatorgehaltes[3] angeordnet. Die maximale SK-Aktivierbarkeit des Kammerwassers bleibt hinter derjenigen des Plasmas zurück, beträgt doch der Agglutinationstiter im analogen Plasmaansatz für den Menschen 1:10000 bis 1:20000, für Katzen und Kaninchen 1:100 bis 1:1000. Die SK-aktivierten Kammerwasser von Mensch und Katze lysieren

[1] Lit. bei E. Deutsch, *Blutgerinnungsfaktoren* (F. Deuticke, Wien 1955).

[2] J. Dausset, Y. Bergerot-Blondel und M. Colin, Vox sanguinis *1*, 95 (1956).

[3] St. Müllertz, *Thrombose und Embolie* (Benno Schwabe, Basel 1955), p. 75.

das Gerinnsel innerhalb 30 min vollständig, Kaninchenkammerwasser teilweise, bei den übrigen ist makroskopisch keine Lyse zu erkennen.

Es ist damit nachgewiesen, dass Kammerwasser von Mensch und verschiedenen Tieren ein fibrinolytisches Fermentsystem enthält, das sich gegenüber SK qualitativ gleich verhält wie das entsprechende Plasma. Es ist ferner wahrscheinlich, dass die aktuelle fibrinolytische Aktivität im Kammerwasser grösser ist als im Plasma.

A. Franceschetti und E. Eichenberger

Universitätsaugenklinik Genf und Forschungsinstitut Dr. A. Wander A.G., Bern, 25. November 1958.

Summary

In the aqueous humour of human subjects and of various animal species, a fibrinolytic system has been demonstrated which, as regards streptokinase, shows the same properties as the corresponding plasma. Presumably the actual fibrinolytic activity in the aqueous humour is greater than in the plasma.

The Incorporation of H^3-Thymidine in Newly Differentiated Nuclei of Roots of *Vicia faba*

Friedkin and Wood[1] have shown that labelled thymidine is readily utilized for desoxyribose nucleic acid (DNA) synthesis, whereas labelled thymine is not, and it has been concluded from biochemical studies that labelled thymidine is incorporated only into the DNA[2,3,4]. H^3-thymidine has been used to investigate, by means of autoradiography, the mode of replication of chromosomes[5,6]. The observations discussed here concern the incorporation of H^3-thymidine into the DNA in nuclei of cells beginning to elongate in roots of *Vicia faba*.

[1] M. Friedkin and H. Wood, J. biol. Chem. *220*, 639 (1956).
[2] P. Reichard and B. Estborn, J. biol. Chem. *188*, 839 (1951).
[3] M. Friedkin, D. Tilson, and D. Roberts, J. biol. Chem. *220*, 627 (1956).
[4] M. Downing and B. S. Schweigert, J. biol. Chem. *220*, 521 (1956).
[5] J. H. Taylor, P. S. Woods, and W. L. Hughes, Proc. nat. Acad. Sci., Wash. *43*, 122 (1957).
[6] L. F. La Cour and S. R. Pelc, Nature *182*, 506 (1958).

Seedlings of *V. faba*, with lateral roots, were grown at a temperature of 20°C in separate containers in 30 ml of tap-water to which 25 μc of H^3-thymidine (supplied by Messrs. Schwartz Inc. New York) was added. After 12 h the roots were washed in water and the plants transferred to pure tap-water. Roots were fixed at 2, 4, 6, 12, and 24 h after the beginning of treatment and autoradiographs of sections and squash preparations were prepared by the stripping film technique.

A surprising feature of the incorporation of H^3-thymidine in *Vicia* roots is the intensive incorporation that occurs in the nuclei of some cells beginning elongation (Fig. 1). Autoradiographs over these nuclei show a greater density than any of those over meristematic nuclei, except in a few apical cells adjacent to the root cap. In roots fixed 2 h after the beginning of treatment, this intense labelling is found in about 25% of the cells at a distance of 3 to 5 mm behind the 1·5 to 2 mm of meristem. It begins in cells at a distance of approximately 1·9 mm from the tip of the root (Fig. 2*a* and 2*b*), as is evident from roots fixed after 2, 4, and 6 h. Some heavily labelled nuclei occur, however, in diminishing frequency as far back as 9 to 10 mm after 6 h, the full length of the available sections.

The intense labelling is at first confined mainly to long columns of nuclei in the cortex, the most heavily labelled nuclei being those furthest from the meristem. Grain counts show an increase in labelling up to 6 h, then the number of grains per nucleus is stable, indicating that the incorporation of H^3-thymidine continues in these nuclei during the first 4 to 6 h. The frequency of labelled nuclei also increase for some time, and after 12 and 24 h the nuclei first to become heavily labelled are found further back in the root, in zones about 15 mm and 30 mm respectively.

In addition to nuclei which are evenly and intensely labelled, a few are found in which labelling is confined to some parts of the nucleus (Fig. 3*a* and 3*b*). This pattern of labelling is usually not connected with nucleolar chromatin or heterochromatic regions.

In all samples of roots a few labelled nuclei occur in elongating cells of the vascular system, but generally only the large obviously polyploid nuclei are labelled.

With exposures curtailed to 4 days, it was possible to make grain counts in autoradiographs of squash preparations of roots fixed after 2 h from the start of treatment. On average about twice as many grains were found over the nuclei at 4–7 mm from the tip than over the most intensely labelled interphase nuclei of the meristem in the same root. These grain counts indicate that in the elon-

Fig. 1.—Incorporation of H^3-Thymidine in a root of *Vicia faba*. Autoradiograph of a section, stained after photographic processing with Toluidine blue.

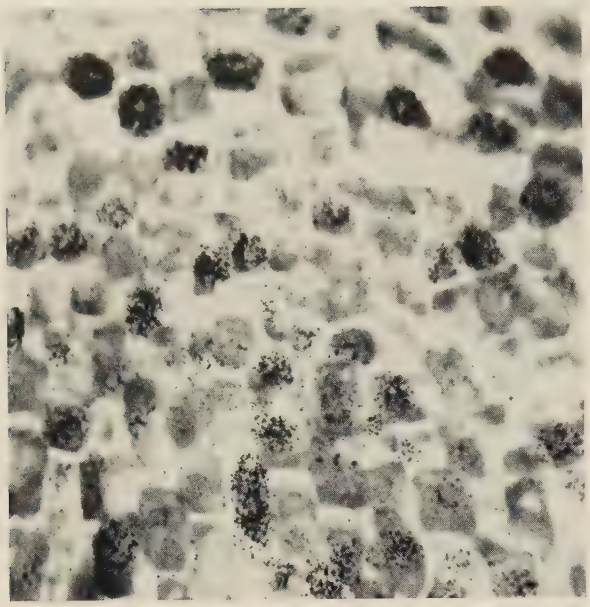


Fig. 2*a*.—Meristematic region of the same section as Fig. 1. Note the heavy autoradiograph over some nuclei in the developing root cap

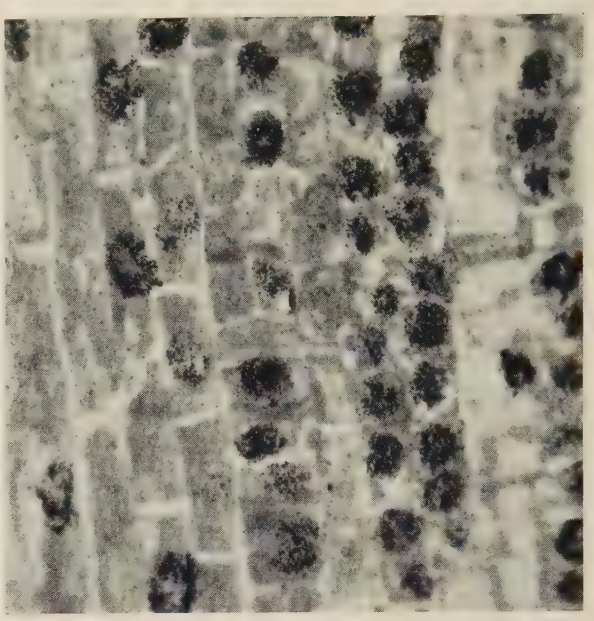
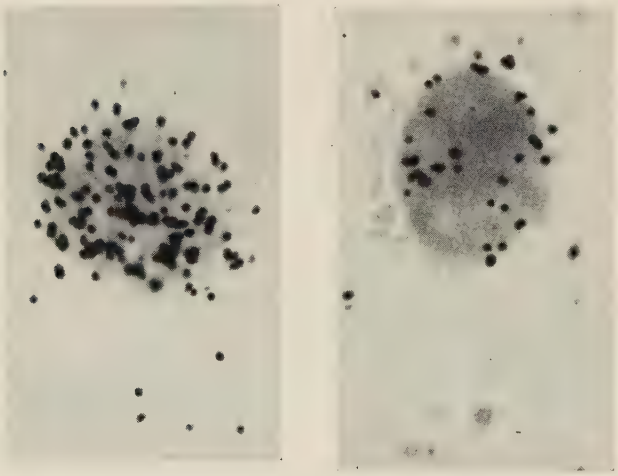


Fig. 2*b*.—Same section as Fig. 1, 1·9 mm from the tip. Note the columns of heavily labelled cells

gating zone adjacent to the meristem, nuclei in the cortex incorporate twice as much thymidine into their DNA as those in meristematic cells. This intensive incorporation appears to begin in nuclei at about 1·9 mm from the tip of the root and coincides with the time at which cell-division ceases.



a *b*

Fig. 3*a*.—A heavily labelled nucleus 2 h after labelling from a squash taken at a distance of 4–7 mm from the tip. Note the even distribution

Fig. 3*b*.— A weakly labelled nucleus from the same squash as Fig. 3*a*. Note the uneven distribution

The amount of DNA per nucleus in roots of *Vicia faba* has been studied by Holmes *et al.*[7], Jensen[8,9], and Deeley *et al.*[10]. The available literature suggests some variation in the length of meristem; our own observations on longitudinal sections agree with those of Jensen[8] whose published data[9] show that nearly all nuclei at a distance of 1·9 mm from the tip of the root contain 4 C (prophase) values of DNA. According to the Feulgen measurements of Deeley *et al.* a small proportion of nuclei however attain an 8 C value at about 8 mm from the tip. Recently Snoad[11], in material grown under the same conditions as those used by us, has found by means of Feulgen-photometry that nuclei in the cortex contain a 4 C amount of DNA at a distance of 2 mm, but that Feulgen values were reduced by 20–25% between 5 and 20 mm distance from the root tip. It can therefore be confidently assumed that no increase in amount of DNA per cell occurs in cortical tissue at a distance of 2 mm from the tip.

Since heavily labelled nuclei are also found in the developing root cap, side by side with meristematic cells, higher specific activity of thymidine is unlikely to be responsible for the heavy labelling in cells in the cortex. It is clear from the available evidence that the incorporation of thymidine into the DNA of cortical cells about to elongate is unconnected with increase in polyteny or polyploidy, and, since intense labelling can be observed as soon as 2 h after treatment, independent of cell division. Such heavily labelled nuclei have not been observed in *Vicia* roots after labelling of DNA with p^{32} [12] or C^{14}-adenine[13]. The incorporation of thymidine in these nuclei has therefore to be regarded as either metabolic exchange of this compound or enrichment of the DNA with thymidine i.e. a change in the base ratios. We feel the latter alternative is unlikely.

At present it would be unwise to regard this phenomenon as a regular prerequisite of differentiation in roots and common to all species. On the other hand, it is reasonable to suspect that the intensive incorporation of thymidine reflects a metabolic activity of the DNA, and therefore possibly of the genetic material, in the cortical

[7] B. E. Holmes, L. K. Mee, S. Hornsey, and L. H. Gray, Exp. Cell Res. *8*, 101 (1955).

[8] W. A. Jensen, Exp. Cell Res. *8*, 506 (1955).

[9] W. A. Jensen, Exp. Cell Res. *10*, 222 (1956).

[10] E. M. Deeley, H. G. Davies, and J. Chayen, Exp. Cell Res. *12*, 582 (1957).

[11] B. Snoad, unpublished.

[12] A. Howard and S. R. Pelc, Exp. Cell Res. *2*, 178 (1951).

[13] S. R. Pelc and A. Howard, unpublished.

nuclei of this region of the root in *Vicia faba*. We think it unlikely that the full specificity of all genes in a nucleus would be required for the function of a differentiating cell at a certain stage, but the amount and localization of the incorporated thymidine indicate that all, or at least most of the DNA is involved. We therefore assume that in this instance the specificity of DNA as a chemical compound is required, though not its possible genetic specificity. It is possible that this incorporation indicates a non-specific action of DNA similar to that reported by ALLFREY and MIRSKY[14]. Whether however the labelling of parts of nuclei in some cells is due to metabolic activity of a few genes or to asynchrony of the chromosomes is less clear.

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Zusammenfassung

Bohnenwurzeln (*Vicia faba*) wurden mit H^3-Thymidin behandelt. Autoradiographien zeigen Aufnahmen von DNS in Zellkernen, welche keine Zellteilungen mehr durchmachen.

[14] V. G. ALLFREY and A. E. MIRSKY, Proc. nat. Acad. Sci., Wash. *43*, 589 (1957).

Über den Einfluss des D,L-Methioninsulfoximins auf den Eiweißstoffwechsel bei Erbsen

Methioninsulfoximin (MSI), ein Derivat der lebenswichtigen Aminosäure Methionin, ist für zahlreiche Organismen von besonderer Giftigkeit. Bereits kleinste Mengen von MSI verursachen bei den Versuchstieren epileptische Krämpfe [1–3]. PACE und DERMOTT[4] bewiesen, dass MSI *in vitro* die Bildung von Glutamin aus Glutaminsäure hemmt; diese Autoren beobachteten auch einen ungünstigen Einfluss des MSI auf die Samenkeimung. Die toxische Wirkung des MSI auf die Versuchstiere kann durch Methionin aufgehoben werden[1,5]. Dieser Befund weist daraufhin, dass MSI (als ein Abkömmling des Methionins) vielleicht auf die Proteosynthese störend einwirkt.

Um den Einfluss von MSI auf den Eiweißstoffwechsel zu prüfen, haben wir als verhältnismässig einfaches Modell die Erbsenkeimlinge in Versuch genommen.

Das benutzte MSI wurde grundsätzlich nach den Angaben von BENTLEY[6] synthetisiert. Zum Versuch wurde der Samen grüner Erbsen (Sorte *Stupicky zeleny*) verwendet. Die Samen wurden in Petrischalen auf Zellstoffwolle, die mit wässerigen Lösungen von MSI (Konzentrationsbereich 10^{-2} bis 10^{-6} *M*) übergossen wurde, gekeimt. Als Kontrollen dienten in destilliertem Wasser gekeimte Erbsen. Nach 10 Tagen wurden die Keimlinge

Tabelle I

Der Einfluss des Methioninsulfoximins auf die Länge der Wurzeln bei den Erbsenkeimlingen

		Wurzeln	
		Anzahl der Pflanzen	Mittelwert der Wurzellänge in mm
Kontrolle		50	43,6
MSI	5×10^{-2} *M*	19*	10,6
	10^{-2} *M*	35	11,8
	10^{-3} *M*	28	20,5
	10^{-4} *M*	24	20,6
	10^{-5} *M*	31	33,0
	10^{-6} *M*	48	34,7

* Bei der Mehrzahl der Samen wurde keine Keimung beobachtet. Alle Werte sind statistisch signifikant.

in die einzelnen Vegetationsorgane (Kotyledonen, Wurzeln, Blätter) zerteilt. Wurzel- und Blattlängen wurden gemessen und abgewogene Mengen des Pflanzenmaterials mit gereinigtem Meersand zerrieben und mit 70%igem Äthanol bei Zimmertemperatur extrahiert. Die Extrakte wurden im Vakuum eingeengt und chromatographisch analysiert. Zur Entfernung anwesender Peptide wurden bestimmte Extraktmengen in eingeschmolzenen Röhren mit zehnfacher Menge 6 *N* HCl 48 h bei 110°C im Trockenschrank hydrolysiert.

Nach Extraktion der freien Aminosäuren und Peptide wurde der Pflanzenrest mit einer Mischung von 6 *N* HCl und 85%iger HCOOH (10:1) bei 110°C innerhalb 48 h hydrolysiert und die erwähnten Hydrolysate im Vakuum von Salzsäureresten befreit.

Zur zweidimensionalen Chromatographie der Aminosäuren, bzw. Peptide in den Extrakten und Hydrolysaten verwendeten wir das Papier Whatman Nr. 1: In erster Richtung im Phenol-Äthanol-Wasser (2:1:1) mit 0,1% Oxin (8-Oxychinolin) in einer Atmosphäre von 3%igem Ammoniak, und in zweiter Richtung in *n*-Butanol-Essigsäure-Wasser (4:1:5), bei zweimal wiederholter Entwicklung[7].

Für die Bestimmung der Hauptformen des Stickstoffs wurden Erbsensamen in ähnlicher Weise gekeimt. In jeder Petrischale wurden 100 Erbsensamen in 30 ml der MSI-Lösung in einer Konzentration 10^{-3} *M* 7 Tage gekeimt. In abgewogenen Mengen vollständig homogenisierter Keimlinge wurden das Trockenmaterial, der Gesamtstickstoff und der Eiweißstickstoff nach BARNSTEIN[8] bestimmt und der Nichteiweißstickstoff als Differenz zwischen Gesamtstickstoff- und Eiweißstickstoffgehalt berechnet.

Proben des Pflanzenhomogenats wurden nach Kjeldahl mineralisiert und der Stickstoff des Mineralisats als Ammoniak mit der Mikrodiffusionsmethode nach CONWAY[9] bestimmt.

Der Versuch mit den Erbsenkeimlingen zeigt, dass MSI das Pflanzenwachstum eindeutig und stark hemmt (siehe Tab. I). In der Konzentration 10^{-2} bis 10^{-3} *M* werden bei den Samen keine Koleoptilen mehr gebildet und die Wurzeln sind deutlich kürzer als bei den Kontrollpflanzen. In der Konzentration 10^{-4} bis 10^{-6} *M* bilden sich noch

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[2] S. N. GERSHOFF, Amer. J. Physiol. *184*, 43 (1956).

[3] Z. LODIN und J. KOLOUŠEK, Československ. Neurologie *19*, 83 (1956).

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[5] Z. LODIN und J. KOLOUŠEK, Československ. Fysiologie, im Druck.

[6] H. R. BENTLEY, E. E. Mc DERMOTT und J. K. WHITEHEAD, Proc. R. Soc., London [B] *138*, 265 (1951)

[7] J. KOLOUŠEK, Naturwissenschaften *44*, 398 (1957).

[8] B. SCHNEIBERG, *Metody chemickych rozboru krmiv* (Bràzda Praha 1951), p. 19.

[9] E. J. CONWAY, *Microdiffusion and Volumetric Error* (London 1947).

Tabelle II

Der Stickstoffgehalt in den Kontrollpflanzen und in den durch Methioninsulfoximin gehemmten Erbsenpflanzen. Alle Werte sind auf Trockengewicht berechnet

		Trockengewicht in %	Gesamt-*N* in %	Eiweiss-*N* in %	Nichteiweiss-*N* in %
Kontrolle		22,10 $s = \pm 0{,}20$	4,26 $s = \pm 0{,}22$	3,25 $s = \pm 0{,}33$	1,02 $s = \pm 0{,}12$
MSI 10^{-3} *M*		28,18 $s = \pm 0{,}20$	4,71 $s = \pm 0{,}37$	4,39 $s = \pm 0{,}32$	0,32 $s = \pm 0{,}25$
Differenz zwischen den Kontroll- und MSI-gehemmten Pflanzen	Kontrolle	100	100	100	100
	MSI 10^{-3} *M*	127,51	110,36	135,41	30,82
	Statistik	FG = 4; $P < 0{,}01$	FG = 4; $P < 0{,}1$	FG = 4; $P < 0{,}01$	FG = 4; $P < 0{,}01$

FG = Freiheitsgrad ($N+N-2$; N = Zahl der Bestimmungen). s = mittlere Abweichung der einzelnen Bestimmung.

Wurzeln und Blätter, die kürzer als bei den Kontrollpflanzen bleiben. Die Hemmung ist in der Konzentration 10^{-6} MSI noch sichtbar.

Die freien Aminosäuren in Erbsen wurden nur soweit berücksichtigt, als sie zum Thema gehörten. Im übrigen wurden sie für das erwähnte Material bereits ausführlich beschrieben[10,11].

Bei zweidimensionaler Papierchromatographie konnten wir zeigen, dass durch die durch MSI hervorgerufene Hemmung Veränderungen nur im qualitativen Bild der freien Aminosäuren der Wurzeln erfolgen. Mit steigender Hemmung sinkt der Gehalt an Asparagin bis zum Verschwinden (Konzentration 10^{-4}). In den Wurzeln der so gehemmten Pflanzen ist der Gehalt an freien Aminosäuren und Peptiden erniedrigt, manche Peptide fehlen sogar völlig. In den Blättern kamen keine Veränderungen des Aminosäurenbildes zur Beobachtung. Bei einer Konzentration 10^{-2} bis 10^{-3} *M* MSI verschwindet Asparagin in den ganzen Pflanzen und auch der Gehalt an verschiedenen Peptiden ist erniedrigt.

Jedoch waren in den sauren Hydrolysaten des Pflanzenmaterials nach Extraktion der freien Aminosäuren und Peptide keine Veränderungen gegenüber den Kontrollen festzustellen.

Der Stickstoffgehalt der Kontrollpflanzen und der mit MSI gehemmten Pflanzen ist in der Tabelle II angegeben.

Als erstes Keimungskriterium des Samens kann das Auftreten des freien Asparagins[12,13] und des Prolins[14] gelten. Bei der durch MSI hervorgerufenen Hemmung sinkt im Gegenteil die Menge des Asparagins stark oder verschwindet ganz. Die Hemmung der Keimung der Erbsensamen durch MSI ist durch eine Vermehrung des Trockengewichtes und des Eiweißstickstoffgehalts sowie durch eine Erniedrigung des Nichteiweißstickstoffgehalts und Verschwinden des Asparagins gekennzeichnet. Alle Befunde zeigen, dass eine steigende Konzentration von MSI eine steigende Hemmung der Proteolyse sowie Proteosynthese zur Folge hat. Würde die Wirkung des MSI nur in der Hemmung der Amidbildung bestehen, dann müsste der Ammoniakgehalt im Gewebe ansteigen, was auch eine Vermehrung des Nichteiweißstickstoffgehaltes verursachte, was aber nicht der Fall ist: der Nichteiweißstickstoffgehalt sinkt ab. Die Menge des Eiweißstickstoffs im Trockengewicht der Versuchspflanzen ist um 35% höher als in den Kontrollpflanzen; was wiederum dafür spricht, dass das MSI eine Hemmung der Proteolyse hervorruft. Auf Grund unserer Befunde kommen wir zur Auffassung, dass es sich beim MSI um einen Hemmstoff der Proteolyse handelt.

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Summary

The influence of the D,L-Methioninesulphoximine on the nitrogen metabolism of germinating seeds has been studied on peas. It has been shown that D,L-Methioninesulphoximine, even at low concentrations, inhibited the germination of seeds and produced a decrease of growth of the roots. The inhibited germinating seeds contained less non-protein-nitrogen and less asparagine than the control plants. On the basis of the experimental results, D,L-Methioninesulphoximine could be considered as an inhibitor of proteolysis.

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[11] A. J. Virtanen, A. M. Berg und S. Kari, Acta chem. scand. *7*, 1423 (1953).

[12] J. Bonner, *Plant Biochemistry*, 1. Aufl. (Academic Press Inc., New York 1950), p. 293.

[13] D. N. Prjanišnikoff, zitiert nach [12].

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Die Serumphosphatasen der Ratte in verschiedenen Lebensaltern

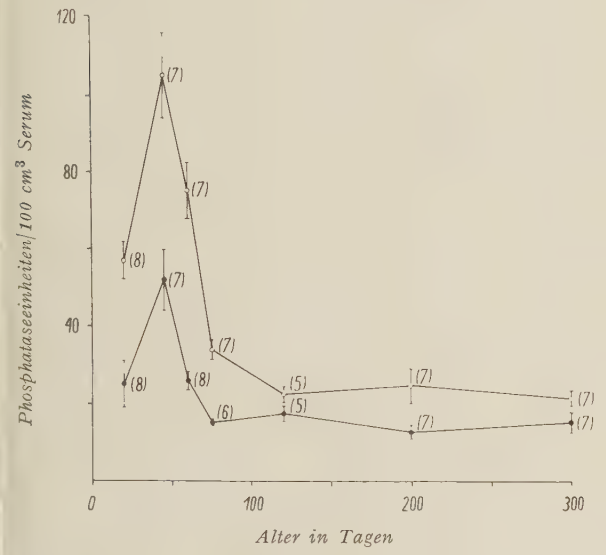
Die alkalische Blut-Serumphosphatase normaler Tiere stammt hauptsächlich aus den Knochen, die saure Serumphosphatase dagegen grösstenteils aus Leber, Milz, Nieren und nur zum kleinern Teil aus den Knochen[1]. Beim Menschen liegen die Aktivitäten beider Serumphosphatasen im Kindesalter hoch und nehmen zur Zeit der Pubertät ab[2]. Gleichzeitig steigt die Ausscheidung der sauren Phosphate im Harn an [3]. Die Schwankungen der alkalischen Phosphataseaktivität werden den Veränderungen der osteoblastischen Tätigkeit zugeschrieben[2]. Diese beim

[1] A. B. Gutman, J. Amer. med. Assoc. *120*, 1112 (1942).

[2] L. C. Clark, E. I. Beck und N. W. Shock, J. Gerontol. *6*, 7 (1951). – R. Emmrich und H. Scheffler, Z. Altersforschung *6*, 156 (1952).

[3] D. Knorr, Klin. Wschr. *36*, 760 (1958).

Menschen beobachteten Aktivitätsveränderungen sollten bei der Ratte, einem Tier mit lebenslänglichem Wachstum, untersucht werden. So wurden 1. die Serumphosphataseaktivitäten bei 52 männlichen Albinoratten im Alter von 20, 45, 60, 75, 120, 200 und 300 Tagen, 2. die Körpergewichtszunahme dieser Tiere und 3. der Einfluss der Kastration auf die Serumphosphatasen geprüft. Bei den etwa 18 h fastenden Tieren sind die Phosphatasen nach der Huggins-Talalayschen Methode[4] wie bereits beschrieben bestimmt worden[5]. Am gleichen Tag wurden jeweils Ratten verschiedener Altersgruppen in den Versuch genommen. Um jahreszeitliche Einflüsse auszuschalten, wurden alle Beobachtungen im Monat Mai durchgeführt.



Die Aktivität der Serumphosphatasen männlicher Ratten in Abhängigkeit vom Alter. o——o = alkalische, •——• = saure Serumphosphatase (Mittelwerte). I = Standardfehler des Mittelwertes. In Klammern Anzahl der Tiere

Phosphataseaktivität und Lebensalter. Die Aktivitäten der alkalischen und sauren Phosphatase (Abb.) wachsen im allgemeinen parallel bis zu einem Maximum im Lebensalter von 45 Tagen an, um danach wieder abzunehmen. Die statitische Auswertung wurde durch den Vergleich des Durchschnittswertes der Altersgruppe von 45 Tagen (höchste Phosphatasenaktivität) mit den durchschnittlichen Werten von allen übrigen Altersgruppen unter Anwendung des «*t*»-Testes durchgeführt. Diejenigen Einzelbeobachtungen (weniger als 4%), bei denen die quadratische Abweichung erheblich mehr als die Hälfte der Summe aller Quadratabweichungen betrug, wurden nach Sealey und Sondern[6] als aberrant angesehen. Die Abbildung enthält diese Werte nicht. Die Aktivitätszunahme der alkalischen Phosphatase vom 20. bis zum 45. Tag ist signifikant ($p < 0{,}01$). Vom 45. bis zum 60. Tag nimmt sie wahrscheinlich signifikant ($p < 0{,}05$) und bis zum 75. Tag stark signifikant ($p < 0{,}001$) ab. Die Aktivitätswerte stabilisieren sich nach dem 120. Tag und zeigen hierauf keine signifikanten Veränderungen mehr bis zum 300. Altertag. Grundsätzlich gilt dasselbe für die saure Serumphosphatase. Sie nimmt vom 20. Tag bis zum 45. Tag auch signifikant zu ($p < 0{,}02$), sinkt dann bis zum 60. Tag hoch signifikant ab ($p < 0{,}001$), um nach Ablauf einer 75tägigen Lebensdauer praktisch unverändert zu bleiben. Zur Zeit des Aktivitätsmaximums ist die Variabilität für beide Phosphatasen am grössten; die Ursache dafür könnte vielleicht darin liegen, dass die verschiedenen Tiere nicht in genau derselben Zeit die höchste Phosphataseaktivität erreichen.

Phosphataseaktivität und Zuwachsrate. Die Zuwachsrate unserer Versuchstiere zeigt gewöhnlich im Alter von 75 Tagen maximale Werte und stimmt nicht mit der Zeit der höchsten Phosphataseaktivität überein. Diesbezüglich sind weitere Untersuchungen notwendig, da die Körpergewichtzunahme nicht als zuverlässiges Kriterium für das Wachstum des Skelettes dienen kann.

Phosphataseaktivitäten und Keimdrüsen. Nachdem die Abnahme der Phosphataseaktivitäten in die Zeit der sexuellen Reifung der Ratte fällt, wurden die Serumphosphatasen bei kastrierten und normalen männlichen, etwa 100 Tage alten Ratten – also zur Zeit, in welcher bei unseren Tieren die Aktivitäten der sauren Serumphosphatase schon stabilisiert sind – bestimmt. Sechs Normalratten zeigten eine saure Phosphataseaktivität von 25,5 ± 3,7 Einheiten (Standardfehler des Mittelwertes). Bei 8 Ratten, die 40 Tage vorher kastriert wurden, stieg die saure Phosphataseaktivität auf 46,0 ± 2,7 Einheiten (hoch signifikant; $p < 0{,}001$) und erreichte damit fast die maximalen Werte unserer Tiere. Interessanterweise wurde die alkalische Serumphosphatase durch die Kastrierung nicht beeinflusst. Zur weiteren Klärung der Frage sollte die Kastrierung in verschiedenen Lebensaltern unternommen werden.

Für die technische Mithilfe sind wir Herrn Gluščević besonders verpflichtet.

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Summary

The serum alkaline and acid phosphatases were examined in male rats between the ages of 20 and 300 days, using the method of Huggins and Talalay[4]. The activities of acid and alkaline phosphatases increase significantly to the age of 45 days, followed by a highly significant decrease up to 120th day for alkaline and to 75th day for acid phosphatase. After castration, highly significant increases in the acid, but no changes in the alkaline serum phosphatase were observed.

Comparison of Choleretic Effects of CYN and Na-Dehydrocholate

1,4 dicaffeylquinic acid (Cynarine or CYN), originally extracted from the large basal leaves of artichoke and later also prepared by synthesis[1], was found to be the active principle of artichoke (Preziosi *et al.*[2–4]).

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Table I

Animal No.	Treatment			Amount of bile in ml ± s.e. secreted during					Percentage variations compared with basal secretion			
	Substance	mg/kg	ml/kg	1st h	2nd h	3rd h	4th h	5th h	2nd h	3rd h	4th h	5th h
12	—	—	—	0·35 ± 0·010	0·31 ± 0·027	0·30 ± 0·021	0·32 ± 0·030	0·29 ± 0·032	– 11·5	– 14,3	– 8·6	– 17·2
8	Ph. B. *M*/5	—	5 % soln.	0·42 ± 0·038	0·38 ± 0·037	0·35 ± 0·061	0·32 ± 0·049	0·29 + 0·033	– 9·6	– 16·7	– 23·9	– 31
5	Na-dehydrocholate	5·53*	0·11	0·48 ± 0·049	0·51 ± 0·055	0·54 ± 0·051	0·48 ± 0·04	0·42 ± 0·023	+ 6·2	+ 12·5	0	– 12·5
8	Na-dehydrocholate	11·85	0·22	0·28 ± 0·029	0·35 ± 0·022	0·39 ± 0·032	0·28 ± 0·014	0·25 ± 0·010	+ 25	+ 39·2	0	– 10·8
9	Na-dehydrocholate	23·7	0·57	0·38 ± 0·028	0·52 ± 0·040	0·39 ± 0·026	0·34 ± 0·019	0·33 ± 0·018	+ 36·8	+ 2·6	– 10·6	– 13·2
9	Na-dehydrocholate	39·5	0·79	0·42 ± 0·040	0·72 ± 0·044	0·38 ± 0·037	0·31 ± 0·072	0·24 ± 0·045	+ 71·4	– 9·6	– 26·2	– 42·9
8	Na-dehydrocholate	59·25	1·19	0·29 ± 0·006	0·64 ± 0·042	0·28 ± 0·035	0·28 ± 0·049	0·20 ± 0·040	+ 120·6	– 3·5	– 3·5	– 31·1
8	Na-dehydrocholate	79	1·58	0·34 ± 0·019	0·78 ± 0·011	0·41 ± 0·017	0·28 ± 0·017	0·25 ± 0·026	+ 129·4	+ 20·5	– 17·7	– 26·5
6	CYN	7	0·14	0·47 ± 0·05	0·50 ± 0·06	0·46 ± 0·07	0·43 ± 0·036	0·45 ± 0·023	+ 6·3	– 2·2	– 8·6	– 4·3
5	CYN	15	0·30	0·46 ± 0·07	0·57 ± 0·09	0·53 ± 0·05	0·42 ± 0·02	0·42 ± 0·04	+ 23·9	+ 15·2	– 8·7	– 8·7
6	CYN	30	0·60	0·31 ± 0·11	0·44 ± 0·06	0·35 ± 0·05	0·29 ± 0·036	0·31 ± 0·11	+ 41·9	+ 12·9	– 6·5	0
5	CYN	50	1	0·42 ± 0·02	0·63 ± 0·05	0·51 ± 0·03	0·39 ± 0·043	0·34 ± 0·012	+ 50	+ 21·4	– 7·2	– 19·1
6	CYN	75	1·5	0·50 ± 0·02	0·96 ± 0·1	0·65 ± 0·06	0·56 ± 0·061	0·50 ± 0·002	+ 92	+ 30	+ 12	0
5	CYN	100	2	0·40 ± 0·01	0·78 ± 0·04	0·60 ± 0·05	0·49 + 0·037	0·44 ± 0·06	+ 95	+ 50	+ 22·5	+ 10

* 5·53 – 11·85 – 23·7 – 39·5 – 59·25 – 79: equimolecular doses to 7 – 15 – 30 – 50 – 75 – 100 mg/kg CYN respectively. s. e. = standard error

CYN possesses choleretic and diuretic properties and has also some interesting effects on cholesterol metabolism.

This paper deals with the results of a series of experiments in which some aspects of the choleretic action of CYN, e.g. dose-effect relation, duration of action, influence on excretion time of bromesulfonephtaleine (B. S. P.) were compared with the same effects exerted by Na-dehydrocholate (NaDC).

Material and methods. Albino rats of the Morini strain weighing 160–220 g were used. Animals of both sexes, but not pregnant females, were employed. CYN[5] and reagent grade NaDC have been used. Both substances were dissolved in phosphate buffer (Ph. B.) *M*/5 (Na_2HPO_4 7·1% solution). With this solvent, a 1–2% solution of CYN may be obtained by heating on a boiling water-bath. These solutions precipitate on cooling. Their pH is about 7 (6·9–7·2). The strength of the CYN and NaDC solutions was calculated in order to inject the required amount of drug in a total volume of 5 ml liquid per kg. Groups of 10–30 rats treated only with Ph. B. (5 ml/Kg), or kept under observation without any treatment, served as controls.

In all our experiments, we used the technique of temporary biliary fistula, described by Preziosi and Loscalzo[2]. In this series of experiments, however, the abdominal wall was sutured after the introduction of the plastic tube into the common biliary duct. Drugs were administered by intravenous injection. The bile flow has been controlled and measured 1 h before and 4 h after the administration of CYN, NaDC or Ph. B. In the controls without any treatment, the flow was recorded for 5 h.

[5] CYN was kindly supplied by Farmitalia.

Results.

1. *Dose-effect relation.* As shown in Table I, CYN may cause a 90% increase of basal biliary flow. For doses up to 50 mg/kg of CYN, as well as for equimolecular doses of

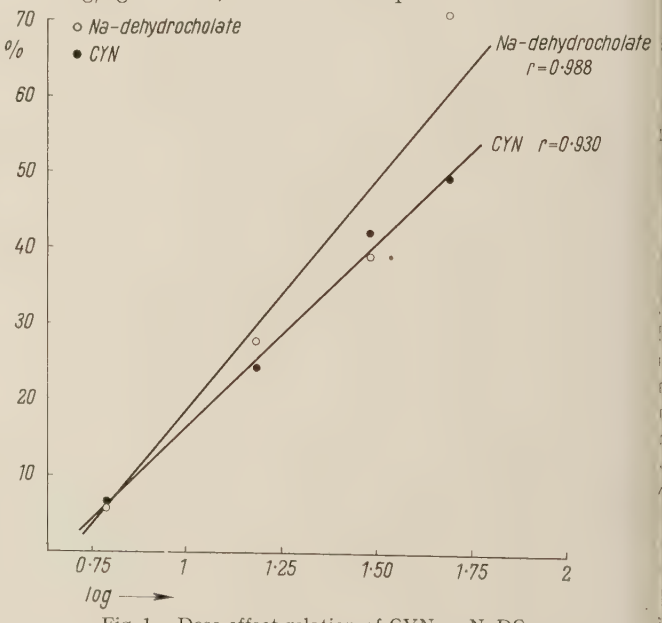


Fig. 1.—Dose-effect relation of CYN or NaDC

The average percentage variations of the biliary flow during the first hour after injection of the drugs has been plotted against the log of the doses. The '*r*' was calculated according to Günther[6]

[6] In G. Poumeau-Delille, *Techniques biologiques en endocrinologie expérimentale chez le rat* (Masson, Paris 1953), p. 161–183.

NaDC, a close dose-effect relation could be observed during the first hour after the injection (Fig. 1).

2. *Duration of action.* The choleretic effect of *lower* doses (7–15 mg/kg) of CYN during the first hour is nearly equivalent to that of equimolecular doses (5·53–11·85 mg/kg) of NaDC, but during the second hour its activity is inferior to that of NaDC. *Medium* doses (30 mg/kg) of CYN and NaDC display about the same choleretic activity during the first and second hours after the injection.

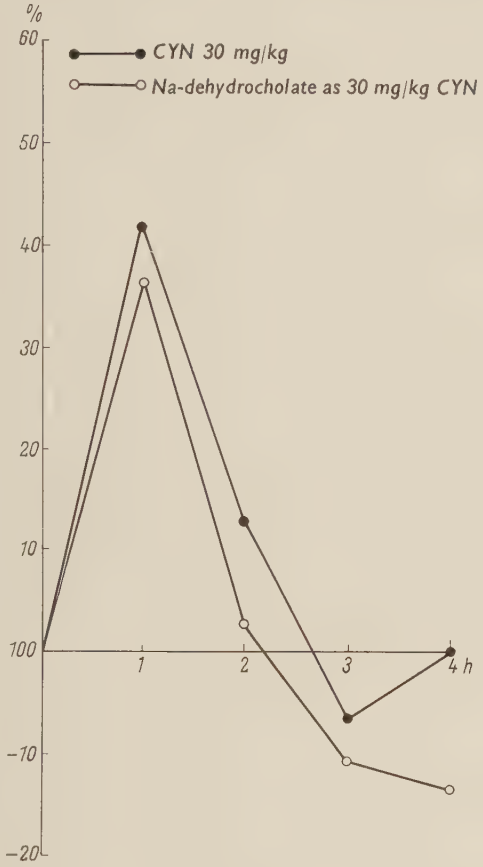


Fig. 2.—Choleresis following different doses of CYN or NaDC
The average percentage variations of the biliary flow with respect to the basal values (basal hour) have been plotted against time (after injection of drugs) during which the observation of biliary flow was performed

During the following hours, a reduction of bile flow may be observed in the animals treated with NaDC, which fails in CYN-treated rats (Table I and Fig. 2). For *higher* doses (50–75 mg/kg), the choleretic activity of CYN during the first hour is slightly inferior to that of equimolecular doses (39·5–59·25 mg/kg) of NaDC, while during the following hours the effect of CYN is more pronounced. During these hours a rather severe reduction of biliary flow below basal values may be observed in NaDC treated rats, but not in CYN-injected animals. The choleretic action of CYN in doses of 100 mg/kg lasts up to 4 h, while the choleretic effect of equimolecular doses of NaDC has a duration of about 2 h (Table I and Fig. 3).

3. *B. S. P. excretion time.* In these experiments B. S. P. (5 mg/kg, 2·5% solution) was injected intravenously 30 min after the injection of CYN or NaDC. The B. S. P. excretion time is not modified or only slightly enhanced by CYN (17–30 mg/kg), as shown in Table II. Equimolecular doses of NaDC do not influence the B. S. P. excretion time.

Table II

Treatment		BSP average excretion time min/s
Substance	mg/kg	
—	—	4 min 45 s
Ph. B. *M*/5 . . .	5 ml/kg	4 min 30 s
NaDC	11·85 mg/kg*	4 min 51 s
NaDC	23·7 mg/kg**	4 min 34 s
CYN	15 mg/kg	3 min 45 s
CYN	30 mg/kg	4 min 12 s

* Equimolecular to 15 mg/kg CYN.
** Equimolecular to 30 mg/kg CYN.

Conclusion and summary. CYN was found to have marked choleretic properties. A close dose-effect relation could be observed during the first hour after injection of the drug up to doses of 50 mg/kg. For the medium doses

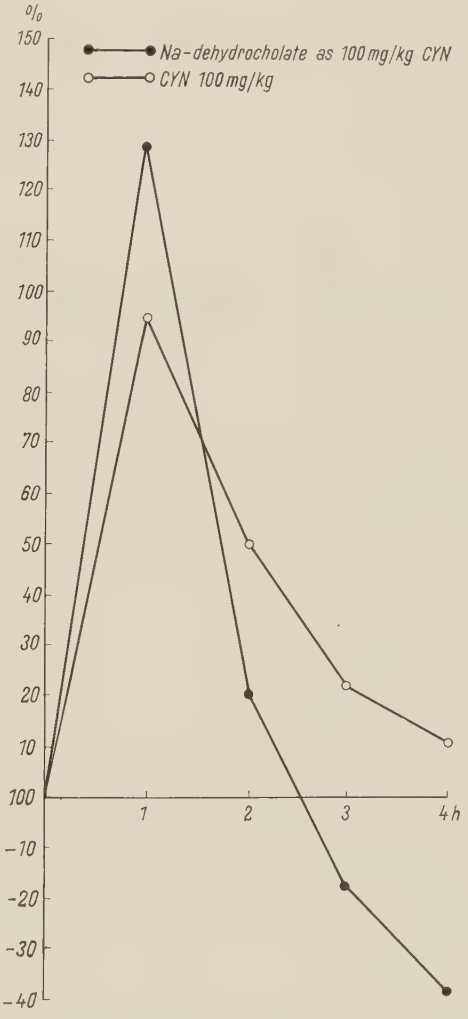


Fig. 3.—Choleresis following different doses of CYN or NaDC
The average percentage variations of the biliary flow with respect to the basal values (basal hour) have been plotted against time (after injection of drugs) during which the observation of biliary flow was performed

the choleretic effect is nearly equivalent to that of NaDC in equivalent doses, while the effect of higher doses was of longer duration, lasting up to 3–4 h. The B. S. P. ex-

cretion time is not modified by CYN in doses equivalent to the doses which may be employed in clinical therapy (Bertolani *et al.*[7]), in contradiction to other choleretics, which may impair the excretory function of the liver (Delcourt[8,9]).

P. Preziosi, B. Loscalzo, and E. Marmo

Department of Pharmacology, University of Naples (Italy), December 1, 1958.

Riassunto

L'attività coleretica, nel ratto, dell'ac. 1,4 dicaffeilchinico (Cinarina) risulta per dosi di 15–30 mg/kg per intensità e durata sovrapponibile a quella del Na-deidrocolato in dosi equimolecolari, per dosi più elevate (75 a 100 mg/kg) più prolungata. La funzione escretrice del fegato non è sfavorevolmente influenzata dalla Cinarina.

[7] F. Bertolani, M. Dardari, and L. Massa, *Significato e valore pratico della utilizzazione di un farmaco ad azione colocrina, l'ac. 1,4-dicaffeilchinico, nella indagine colangiocolecistografica con mezzo di contrasto endovenoso (ricerca sperimentale e clinica)* (Casa Ed. Ambrosiana, Milano 1957).

[8] A. Delcourt and A. Domb, J. Physiol. *48*, 494 (1956).

[9] A. Delcourt, Ann. Soc. R. Sci. méd. natur. Bruxelles *9*, 281 (1956).

Phenothiazine Derivatives as Inhibitors of the Glucose Oxidative Pathway in Human Erythrocytes

It has been previously noted that human erythrocytes in the presence of methylene blue oxidize glucose by way of phosphogluconate oxidation[1]. The oxidation of glucose is increased 20 to 50 times over the low level established without the dye. The enzymes necessary for the oxidation of glucose to ribose-5-phosphate are present in the erythrocyte[2]. The oxidative steps of the pentose phosphate pathway, because of the ability to produce TPNH[3], have an important function in cell growth and synthesis[4].

In view of the above, and because of an interest in the effects of tranquilizing agents on enzyme systems, the behaviour of three phenothiazine derivatives on phosphogluconate oxidation in erythrocytes was investigated.

Human blood, collected in acid citric-dextrose (Formula B, National Institutes of Health) was obtained from the local Red Cross. The cells were prepared according to Huennekens *et al.*[5]. Since there is a loss in the ability of the cells to oxidize glucose when they are stored several weeks, without any decrease in hexokinase activity[6], adenosine (2100 μM/100 ml of cells) was added prior to storage of the erythrocytes at 4°C. Adenosine triphosphate declines during storage and is regenerated upon the addition of adenosine[5].

[1] O. Warburg, F. Kubowitz, and W. Christian, Biochem. Z. *227*, 245 (1930).

[2] F. Lipmann, Nature *138*, 588 (1936).

[3] TPN – Triphosphopyridine nucleotide; TPNH – reduced TPN.

[4] B. L. Horecker and H. H. Hiatt, New England J. Med. *258*, 225 (1958).

[5] F. M. Huennekens, L. Lieu, H. P. A. Myers, and B. W. Gabrio, J. biol. Chem. *227*, 253 (1957).

[6] B. W. Gabrio, C. A. Finch, and F. M. Huennekens, Blood *11*, 103 (1956).

Glucose oxidation in whole cells was measured by the standard Warburg technique. The system as employed by Huennekens[5] was used. The phenothiazines were added at the expense of the Krebs-Ringer phosphate solution. Both cells and drugs were added initially to the main compartment of the Warburg flask and were incubated together 15 min before starting the experiment.

Effect of Inhibitors on Enzyme Activity

Drug	Conc. $\times 10^{-4}$ M	% Inhibition
Promazine	5·0	16·0
	2·5	10·0
	1·25	1·4
SKF 5883	5·0	48·8
	2·5	25·5
	1·25	13·8
Adazine	5·0	51·3
	2·5	21·8
	1·25	12·3

The Table indicates the inhibiting effects of the three phenothiazines studied. At equivalent molar concentrations, adazine [10-(3-dimethylaminopropyl)-2-trifluoromethyl phenothiazine hydrochloride] was the most effective in preventing oxygen consumption by the cells followed by SKF 5883 (N,N-dimethyl-10-[3-(1-methyl-4-piperazinyl)-propyl]-2-phenothiazine sulfonamide dimethanesulfonate) and promazine [10-(3-dimethylaminopropyl)-phenothiazine hydrochloride]. It was also observed that the inhibiting effect of drugs increased with increasing concentration of the drug. These results resemble those found in other systems[7].

Effect of Drug Combination on Enzyme Activity
A = Promazine at $2{\cdot}5 \times 10^{-4}$ M;
B = SKF 5883 at $2{\cdot}5 \times 10^{-4}$ M;
C = Combination of the two, each at $2{\cdot}5 \times 10^{-4}$ M

Additional observations were made in which two of the drugs were present in the same reaction mixture at $2{\cdot}5 \times 10^{-4}$ M. As indicated in the Figure, these drugs do

[7] E. W. Helper, M. J. Carver, H. P. Jacobi, and J. A. Smith, Arch. Biochem. Biophys. *76*, 354 (1958).

not have an additive effect but inhibit glucose oxidation to a value intermediate between those obtained when the drugs are used alone at these concentrations. Similar results were obtained with other combinations.

Previous work has indicated that some of these compounds inhibit cytochrome oxidase from rat liver and brain [7]. Reduced triphosphopyridine nucleotide is oxidized slowly by this system. However, TPNH oxidase is present in the erythrocyte and may possibly be the specific enzyme inhibited by these compounds. These experiments were not designed to establish this.

The molar concentrations required in this study were in excess of the pharmacological dose. However, it is possible that the inhibition noted may be the basis for other clinical effects of the drugs. Further work is in progress on the use of human red blood cells in studying enzyme inhibition by phenothiazine derivatives.

Investigation supported in part by Nebraska Fund for Psychiatric Research and Grant M-2204, National Institute of Mental Health, Public Health Service[8].

M. J. Carver and Nadine Roesky

Neurochemical Laboratory, Nebraska Psychiatric Institute, College of Medicine, Omaha (Nebraska), December 1, 1958.

Zusammenfassung

Phenothiazinabkömmlinge unterscheiden sich in ihrer Wirksamkeit als Hemmstoffe im oxydativen Glukosestoffwechsel in menschlichen Erythrozyten. Kombiniert man verschiedene Phenothiazinderivate, so erhält man einen mittleren Hemmungsgrad, der zwischen jenen liegt, die man bei Anwendung der einzelnen Wirkstoffe für sich allein erhalten würde. Somit erscheint es nicht wahrscheinlich, dass Hemmung des oxydativen Glukosestoffwechsels die prinzipielle Wirkungsweise der Phenothiazinabkömmlinge darstellt.

[8] SKF-5883 was kindly supplied by Smith, Kline and French Laboratories, Philadelphia; Promazine by Wyeth Laboratories, Philadelphia; and Adazine by the Upjohn Company, Kalamazoo.

The Extractibility of Calf Thymus Histone Fractions

It is well known that the calf thymus histone is composed of at least two main fractions. The arginine-rich fraction contains approximatively five times more tyrosine than the lysine-rich one [1–4]. The difference in the solubility of the sulphates of these two main fractions of calf thymus histone was used by some authors for its fractionation [4,5].

The nuclear material was prepared by modified extraction of the cytoplasm with 0·14 *M* NaCl according to Butler *et al.* [6]. Histone was extracted with diluted hydrochloric acid in two ways: in the first, the concentration of the acid, when mixed with nuclear material, amounted to 0·1 *N*; in the second to 0·2 *N*. Apart from this method, histone was isolated by extraction of the bulk of proteins

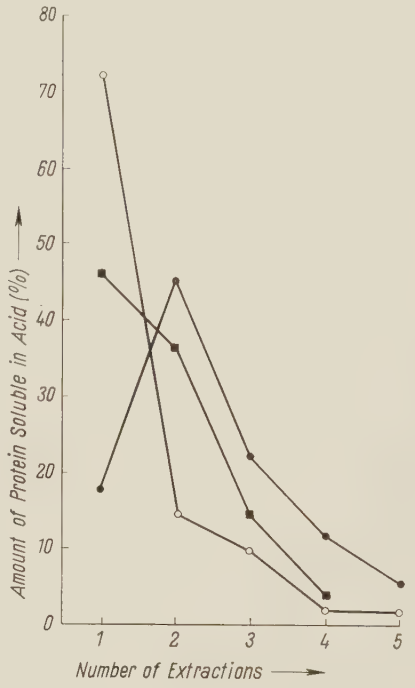


Fig. 1.—The amount of acid-soluble protein (histone) in subsequent extractions of nuclear material, prepared by using 0·14 *M* NaCl extracted with 0·1 *N* HCl (●—●—●) and 0·2 *N* HCl (o—o—o) and of the material prepared according to Sevag *et al.* [7] and extracted with 0·1 *N* HCl (■—■—■)

remaining after isolation of desoxyribonucleic acid (DNA), according to Sevag *et al.* [7]. This protein-containing material was also extracted with 0·1 *N* hydrochloric acid. For the complete removal of all the protein soluble in diluted hydrochloric acid, five extractions are necessary (Fig. 1).

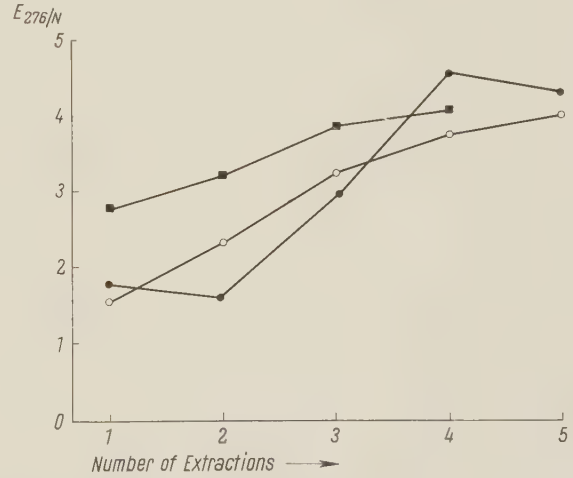


Fig. 2.—The E_{276}/N ratios of subsequent extractions of calf thymus histone. Histone extracted with 0·1 *N* HCl from the nuclear material prepared by using 0·14 *M* NaCl (●—●—●), histone prepared from the same material extracted with 0·2 *N* HCl (o—o—o) and histone extracted with 0·1 *N* HCl from the proteins resulting from the Sevag process (■—■—■)

[1] P. F. Davison and J. A. V. Butler, Biochim. biophys. Acta *15*, 439 (1954).

[2] M. M. Daly and A. E. Mirsky, J. gen. Physiol. *38*, 405 (1955).

[3] Ch. F. Crampton, S. Moore, and W. H. Stein, J. biol. Chem. *215*, 787 (1955).

[4] N. Ui, Biochim. biophys. Acta *25*, 493 (1957).

[5] E. Stedman and E. Stedman, Philos. Trans. Soc. [B] *235*, 565 (1951).

[6] J. A. V. Butler, P. F. Davison, D. W. F. James, and K. V. Shooter, Biochim. biophys. Acta *13*, 224 (1954).

[7] M. G. Sevag, D. B. Lackman, and J. Smolens, J. biol. Chem. *124*, 425 (1938).

The content of the aromatic amino acids, especially tyrosine, in the lysine-rich fraction of the calf thymus histone being several times lower than the content of the arginine rich fraction, the ratio between optical density (measured at 276 mμ – density maximum of histone – in 10 mm silica cells) and nitrogen content of the solution could be used as criterion for fraction distribution in samples of isolated histones. Growth of the value of this ratio in individual samples, obtained by successive acid extractions, was observed, following every subsequent extraction. This indicates that in the first extraction the tyrosine-poor (lysine-rich), in the last extractions the tyrosine-rich (arginine-rich) fraction of histone predominates. In Figure 2 these ratios for different types of isolation are expressed graphically.

The content of aromatic amino acids in single extractions obtained by the method described above, expressed as the ratio between E_{276} and N, seems to suggest the stronger binding of arginine – and tyrosine – rich fraction on DNA. Similar results have been obtained by dissociation of different histone fractions from calf thymus deoxyribonucleoprotein in changing the concentration of $Ba[OAc]_2$ and ethanol[8].

The extraction with 0·1 *N* HCl is more specific for preparing calf thymus histones richer in one of the two main fractions (Fig. 1, 2). The fractionating effect of the extraction with diluted sulphuric acid was used for preparation of the two main fractions of calf thymus histone by Ui[4], but the use of the SO_4 ions brings about the aggregation of arginine-rich histones and therefore it is better to replace these by the Cl ions.

By the extraction of the protein-containing material prepared according to Sevag *et al.*[7] from calf thymus nuclei with 0·1 *N* HCl, the greater part of the acid-soluble protein is extracted by the first and by the second extraction. The fractionation effect of this subsequent extraction is therefore very low. In course of the preparation of the deoxyribonucleoprotein by extraction of calf thymus nuclei with 1 *M* NaCl, or in course of the treating of this deoxyribonucleoprotein with the mixture of chloroform and *n*-butanol (according to Sevag *et al.*), part of the lysine-rich (tyrosine-poor) fraction disappeared, as can be seen in Figure 2.

L. Hnilica

Oncological Research Institute, Bratislava (Czechoslovakia), November 24, 1958.

Zusammenfassung

Bei der Extraktion von Zellkernen mit Hilfe verdünnter Salzsäure ändert sich die Zusammensetzung einzelner Extrakte in Abhängigkeit von der Konzentration der benutzten Säure. Die Fraktion des Kalbsthymushistons, die an Arginin und Tyrosin reich ist, scheint an die Desoxyribonukleinsäure des Zellkernes fester gebunden zu sein als die zweite, an Tyrosin arme und an Lysin reiche Fraktion.

[8] Ch. F. Crampton, J. biol. Chem. *227*, 495 (1957).

Action d'un hépatome ascitique du rat sur les acides nucléiques du pancréas et du rein[1]

Au cours de travaux antérieurs[2,3], nous avons étudié l'effet de la présence d'un hépatome ascitique sur les acides ribo- et désoxyribonucléiques (A.R.N. et A.D.N.) du foie et des surrénales du rat. Il s'est avéré que l'incorporation du ^{32}P dans l'A.R.N. et la quantité absolue de ce constituant augmentent aussi bien dans le foie que dans les surrénales. Un accroissement de la biosynthèse de l'A.D.N. a été observé seulement dans le tissu hépatique. Il nous a paru intéressant de rechercher dans des conditions expérimentales similaires les modifications éventuelles au niveau du pancréas et du rein.

Nos essais ont porté sur des rats blancs de souche Wistar groupés en lots du même sexe et de la même portée. Dans chaque lot, la moitié des rats a reçu une injection intrapéritonéale de 1 cm^3 de liquide d'ascite qui provoque l'apparition d'une abondante ascite[4] au bout de 5 jours. Les rats sont alors sacrifiés par saignée 24 h après l'injection intra-musculaire de 100 μc de ^{32}P par 100 g de poids corporel.

Les organes sont prélevés rapidement, pesés et traités selon les techniques décrites ailleurs pour la détermination de la quantité d'A.R.N. et d'A.D.N., de leurs activités spécifiques et de celle du PO_4 acido-soluble[2].

Les essais concernant les modifications au niveau du pancréas ont porté sur 46 rats groupés en 5 lots, dont 23 témoins. Pour le rein, nous avons effectué 6 expériences sur 24 rats dont 12 témoins.

Les résultats de nos essais sont résumés dans les graphiques I et II.

Nous y avons consigné les quantités de protéines, de phosphore ribonucléique, de phosphore désoxyribonucléique, l'activité spécifique (A. S.) du PO_4 acido-soluble, l'activité du ^{32}P incorporé dans l'A.R.N. rapportée à l'A.S. du PO_4 ainsi que l'A.S. de l'A.D.N. pour le pancréas. Nous n'avons pas présenté les valeurs d'A.S. de l'A.D.N. du rein qui sont très faibles chez les témoins comme chez les porteurs d'hépatomes. Pour l'expression de l'incorporation du ^{32}P dans l'A.R.N., il nous a paru utile de tenir compte de la quantité totale du ^{32}P retrouvé dans l'A.R.N. et de la rapporter à l'A.S. du PO_4 acido-soluble du tissu car ainsi la comparaison entre témoins et animaux en expérience est plus rigoureuse.

Il ressort de l'examen des graphiques que, dans le cas du rein, on n'observe aucune variation des quantités de protéines, d'A.D.N. et du quotient

$$\frac{^{32}P \text{ incorporé dans l'A.R.N.}}{\text{A. S. } PO_4}$$

chez les animaux porteurs de tumeur. En ce qui concerne la quantité d'A.R.N., les différences ne sont pas significatives. Au niveau du pancréas les quantités absolues de protéines et d'A.R.N. restent également inchangées alors

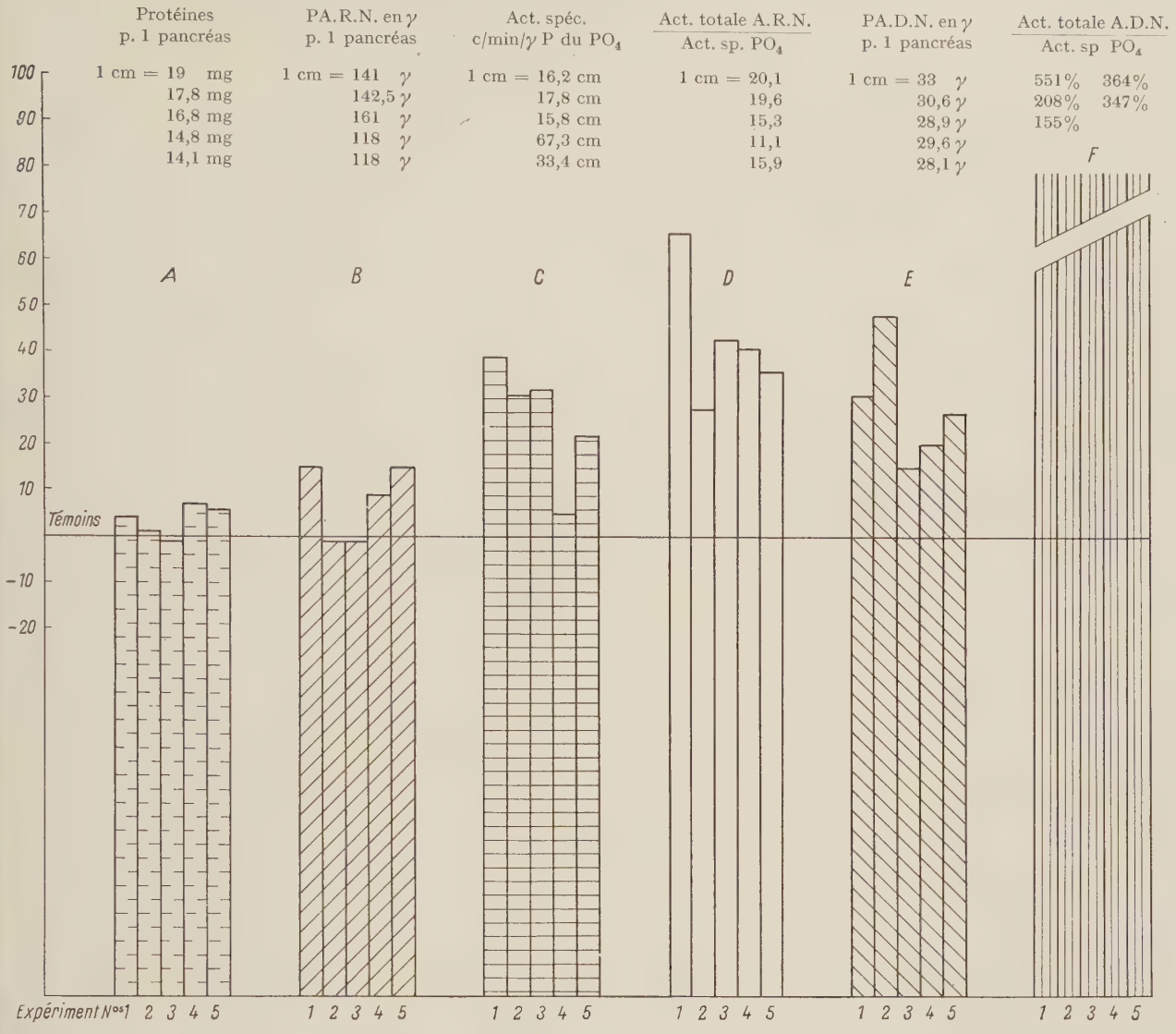
[1] Travail effectué avec l'aide matérielle de l'Institut National d'Hygiène.

[2] M. Wintzerith, L. Mandel et P. Mandel, C. R. Soc. Biol. *151*, 2199 (1957).

[3] L. Mandel, M. Wintzerith et P. Mandel, J. Physiol. *50*, 401 (1958).

[4] Nous devons la souche d'hépatome ascitique à l'obligeance de M. Zajdela de la Fondation Curie, que nous remercions très vivement.

Fig. 1. Influence d'un Hépatome Ascitique sur les Acides Nucléiques du Pancréas chez le Rat blanc



Graphique 1. La ligne horizontale à 100 mm de l'axe des abscisses sur le graphique original, représente les valeurs des témoins, les colonnes les valeurs relevées chez les animaux porteurs de tumeur. Chaque colonne correspond à une expérience portant sur 9 rats. L'échelle permettant de retrouver les valeurs expérimentales est consignée au-dessus de chaque colonne. La hauteur de la colonne au-dessus de la ligne horizontale fournit les différences en pour cent par rapport aux témoins.
Les valeurs de protéines (*A*), d'acide ribonucléique (*B*) et d'acide désoxyribonucléique (*E*) expriment les quantités absolues pour 1 pancréas. Le groupe de colonnes *D* et *F* exprime le nombre de coups/min de la totalité de l'acide ribonucléique (*D*) et de l'acide désoxyribonucléique (*F*) soit la totalité de ^{32}P incorporé rapportée à l'activité spécifique du PO_4 acido-soluble.

que la quantité totale de ^{32}P incorporé dans l'A.R.N. et l'A.D.N. rapportée à l'A.S. du PO_4 acido-soluble est nettement augmentée. Notons que dans aucun cas, nous n'avons trouvé de métastase.

Ainsi, parmi les divers organes que nous avons étudiés, on peut distinguer deux catégories, d'une part le pancréas ainsi que le foie et les surrénales étudiés antérieurement[2,3], qui manifestent un accroissement net de l'activité métabolique de l'A.R.N. et même de l'A.D.N., d'autre part le rein qui ne subit aucun changement.

Nous avons déjà attiré antérieurement l'attention sur la labilité de l'A.R.N. et de l'A.D.N. du pancréas et des surrénales[5]. De même l'activité métabolique de l'A.R.N. du foie est très supérieure à celle du rein; il semble donc que la présence d'une tumeur se manifeste avant tout au niveau des tissus prédisposés naturellement à une grande activité métabolique.

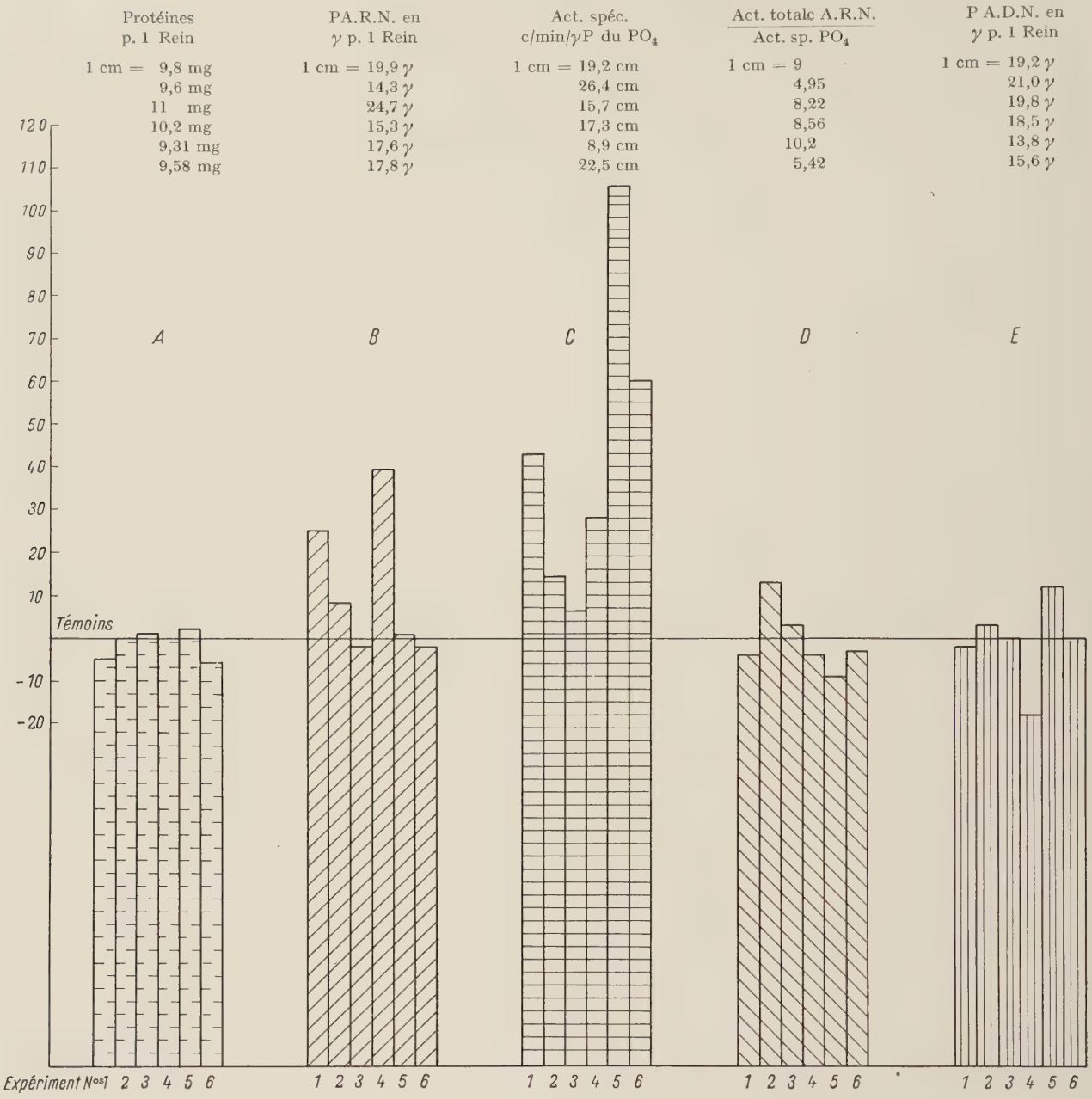
Kelly *et al.*[6] avaient déjà signalé un accroissement de l'A.S. de l'A.D.N. chez des souris porteuses d'un carcinome mammaire et chez des rats auxquels on avait greffé des lymphosarcomes. De même, Khouvine[7] avait constaté une augmentation de l'A.S. de l'A.R.N. du foie chez des animaux porteurs d'un épithélioma atypique. Diverses hypothèses pourraient être invoquées pour expliquer les phénomènes observés par les autres auteurs[6,7] et par

[5] P. Mandel, M. Jacob et L. Mandel, C. R. Acad. Sci., Paris *238*, 288 (1954).

[6] L. S. Kelly, A. H. Payne, M. R. White et H. B. Jones, Cancer Res. *11*, 694 (1951).

[7] M. Mortreuil et Y. Khouvine, Bull. Soc. Chim. biol. *39*, 161 (1957).

Fig. 2. Influence d'un Hépatome Ascitique sur les Acides Nucléiques du Rein chez le Rat



Graphique 2. La ligne horizontale à 100 mm de l'axe des abscisses sur le graphique original, représente les valeurs des témoins, les colonnes les valeurs relevées chez les animaux porteurs de tumeur. Chaque colonne correspond à une expérience portant sur 4 rats. L'échelle permettant de retrouver les valeurs expérimentales est consignée au-dessus de chaque colonne. La hauteur de la colonne au-dessus de la ligne horizontale fournit les différences en pour cent par rapport aux témoins.

Les valeurs de protéines (*A*), d'acide ribonucléique (*B*) et d'acide désoxyribonucléique (*E*) expriment les quantités absolues pour 1 rein. Le groupe de colonne *D* exprime le nombre de coups/min de la totalité de l'acide ribonucléique (*D*) soit la totalité de ^{32}P incorporé rapportée à l'activité spécifique du PO_4 acido-soluble.

nous-mêmes[2,3]. L'idée d'une substance qui stimule les biosynthèses ou rompt l'équilibre métabolique normal au niveau d'organes sensibles, paraît devoir être envisagée en premier lieu. Des essais d'isolement d'une telle substance sont restés jusqu'à présent sans succès, ce qui n'exclut pas son existence.

M. Wintzerith, N. Klein, L. Mandel et P. Mandel

Institut de Chimie biologique, Faculté de Médecine, Strasbourg, le 13 décembre 1958.

Summary

Animals having an ascites hepatoma show an increase in the incorporation of P^{32} into ribonucleic acid and desoxyribonucleic acid of the pancreas, whereas no change is seen in the kidneys. The variations noted in the pancreas may be compared with those observed previously in the liver and the adrenals. It can therefore be presumed that a substance which stimulates biosynthesis or disrupts the normal metabolic balance is produced by the ascites cells.

Particulate-Bound Pigment of *Serratia marcescens* (*Chromobacterium prodigiosum*)

The red pigments produced by the microorganism, *Serratia marcescens*, are usually regarded as being cellular and water insoluble[1]. However, some reports have stated that the pigments can diffuse from the cells, and thus might be regarded as being water soluble[2]. Monk[3] reported spectral studies carried out on a pigmented extract obtained from *S. marcescens* cells disrupted in a Mickle disintegrator. Similar preparations were made by Linnane and Still[4] employing a high-speed mechanical disintegrator. The latter investigators reported that the pigment was isolated as a protein complex. However, no details concerning the complex were given in their report. The present communication extends the observations of Monk[3] and of Linnane and Still[4], and presents evidence that the red pigment obtained from mechanically disrupted cells is bound to small particles.

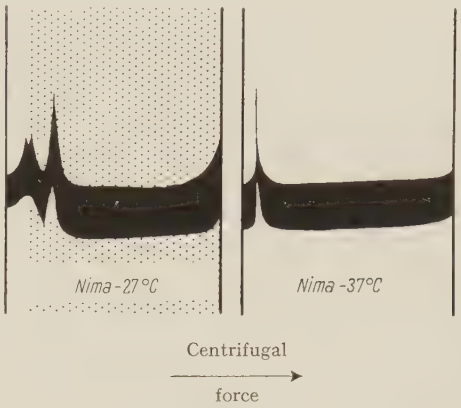
S. marcescens strain Nima was grown for 5 days on an agar medium[5]. The organisms were suspended in distilled water, and disrupted in a high-speed mechanical shaker[6]. Clarification of the resultant turbid material was carried out by step-wise centrifugation. The sediment remaining after each centrifugation was pigmented, but the supernatant remaining after treatment at $50\,000 \times g$ for 1 h was still a deeply pigmented, red color. This material showed no visible turbidity. However, when it was centrifuged at $105\,000 \times g$ for 2 h all of the pigment sedimented as a sharply circumscribed, red pellet. The supernatant was completely devoid of pigment.

The red pellet was washed, resuspended in distilled water, and analyzed in the spectrophotometer. In the visible region, the spectrum was identical to that reported by Monk[3], and showed maximum optical density at 495 mμ. A shoulder was present at 530 mμ, and the minimum was at 425 mμ. Monk[3] did not report the ultraviolet spectrum of his material. Our preparation exhibited a maximum peak in the ultraviolet region at 260–270 mμ. This absorbance probably represents protein and nucleic acid material present in the suspension.

Chemical tests carried out on the material established the presence of protein, lipid, carbohydrate, and nucleic acid substances. The pigment could not be dialyzed from the suspension, nor could it be separated from the sediment by paper electrophoresis. The latter procedure did not move the sediment.

In order to demonstrate the association of pigment with the sedimenting material, the organism was grown at 37°C. At this temperature no pigment was produced, and centrifugation at $105\,000 \times g$ of material prepared as outlined above produced a viscous, straw-colored sediment that did not show a sharp separation from the clear supernatant.

Washed preparations of material obtained from pigmented and colorless cells were examined in an analytical ultracentrifuge. The results are presented in the Figure. Pigmented material demonstrated three fractions, and the peak of one component coincided exactly with the pigment front. A second component sedimented more slowly than the pigment fraction while the third fraction was present within the pigmented area. The preparation obtained from colorless organisms exhibited only one component which appeared to be a slow-moving fraction when compared to the sedimentation pattern of the pigmented material. However, exact comparison between the two samples could not be carried out because the presence of pigment probably altered the sedimentation constants of the 27°C components.



Tracings of analytical ultracentrifuge patterns of $105{,}000 \times g$ preparations obtained from *Serratia marcescens* organisms grown at 27°C (pigmented) and at 37°C (colorless). The pictures were taken after the apparatus had reached a speed of 50,740 r.p.m. The stippled area represents the red pigment. Sedimentation proceeds to the right

Electron microscopic examination of washed material prepared from the sediment of pigmented organisms showed the preparation to be heterogenous and to consist of several sizes and shapes of particles. Similar material obtained from colorless organisms was homogeneous, and consisted of small, round particles.

Centrifugal studies have been carried out on cellular material obtained from several other *Serratia* strains which produced pigments bound to the cells. In all cases, an apparently clear red material prepared by mechanical disruption of the cells yielded a red pellet and colorless supernatant when centrifuged at $105\,000 \times g$ for 2 h.

The data presented indicate that the pigment obtained by mechanical disruption of certain strains of *S. marcescens* is bound to cellular particles. Similar associations between bacterial pigments and subcellular particles have been reported for *Streptococcus pyogenes*[7], *Corynebacterium* species[8], and *Rhodospirillum rubrum*[9]. The particles of the latter organism apparently function in the photosynthetic mechanism of the organism, but the function of the substances in the other two organisms is unknown. As has been demonstrated in *Serratia*, colorless strains of *Corynebacterium* species or *R. rubrum* lack particles similar to those associated with pigmented organisms.

The origin of the particles demonstrated in *Serratia*, as well as their function, and the nature of the bound pig-

[1] G. S. Wilson and A. A. Miles, *Topley and Wilson's Principles of Bacteriology and Immunity*, 4th Ed. (Williams and Wilkins, Baltimore 1955), p. 730.

[2] R. S. Breed, E. G. D. Murray, and N. R. Smith, *Bergey's Manual of Determinative Bacteriology*, 7th Ed. (Williams and Wilkins, Baltimore 1957), p. 360.

[3] G. W. Monk, J. Bacteriol. *74*, 71 (1957).

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ment must still be elucidated. Monk[3] speculated that the orange fraction of *Serratia* pigment[5] might be bound to intracellular particles and to be of importance in cellular metabolism.

This investigation was supported by research grant E-670(C3), and by a Medical Student Summer Fellowship (W.W.T.) from the National Institutes of Health, United States Public Health Service.

W. W. Taylor and R. P. Williams

Department of Microbiology, Baylor University College of Medicine, Houston (Texas), October 21, 1958.

Résumé

Le broyage mécanique de *Serratia marcescens* pigmentés produit un liquide rouge clair. Après centrifugation à 105000 × *g*, le pigment se sédimente sous forme d'un agglomérat rouge et la phase liquide reste incolore. La courbe du matériel de l'agglomérat remis en suspension montre à l'ultracentrifuge un seul sommet coïncidant avec le pigment. *S. marcescens* non pigmentés ne produisent pas d'agglomérat après broyage et centrifugation et donnent une courbe différente à l'ultracentrifuge.

Effect of Insulin on Potassium Transfer in Human and Chicken Erythrocytes[1]

Several observations suggest that insulin may increase the permeability of a variety of cell types to a variety of substances. For example, insulin stimulates the penetration of glucose[2] and of amino acids[3,4] into muscle cells and the efflux of aldolase from skeletal muscle[5], and modifies the membrane potential of muscle fibers[6]. In addition, it has been shown that intracellular potassium increases in liver, muscle, erythrocytes, and brain during hypoglycemia[7–11]. The experiments described in this paper were carried out to study if insulin modifies the loss of potassium from erythrocytes during cold storage and its partial re-entry following incubation at 37°C.

Blood samples were obtained from the antecubital vein of 4 normal human subjects, 1 untreated patient in diabetic coma, and from the heart of 4 normal white Leghorn chickens. The needle was removed from the syringe and the blood transferred carefully to flasks containing powdered heparin[12] and glucose (10 mg/ml) and stored at about 4°C. Agitation was avoided to minimize hemolysis and aseptic technic was used throughout. After 6 days of cold storage, 5 or 6 5 ml aliquots of each sample of blood were incubated in a Dubnoff shaker at 37°C moving at the rate of 50 oscillations/min, in an atmosphere of 95% O_2 and 5% CO_2. Ouabain[13] ($7{\cdot}2 \times 10^{-6} M$ in 0·1 ml) and/or glucagon-free insulin[14] (1 u in 0·1 ml) were added to the samples as indicated. Potassium was measured in the plasma with a Coleman flame photometer before and after incubation and the potassium uptake by the cells calculated by difference, according to the method of Kahn and Acheson[15]. The volume of cells was measured by means of hematocrit determinations before and after incubation, in duplicate.

Table II

Effect of insulin and ouabain on potassium uptake by incubated human erythrocytes previously stored at 4°C for 6 days. Incubation time: 3 h at 37°C

	No. of experiments	Potassium Uptake (mEq/l of cells ± S.D)
Controls	4	+3·1 ± 0·76
Ouabain	4	−0·6 ± 0·35
Ouabain + Insulin .	4	−1·3 ± 0·34

Table I shows that normal human erythrocytes removed measurable quantities of potassium from the incubation medium, that the potassium uptake of the erythrocytes of a patient in diabetic coma was appreciably smaller and that the uptake of the nucleated red cells of the chicken was appreciably greater than that of normal human red cells. Insulin had no measurable effect on potassium uptake under the conditions of these experiments. Table II shows that ouabain blocked potassium uptake by incu-

[1] Aided by a grant from the Chicago Heart Association.

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[3] M. E. Krahl, J. biol. Chem. *200*, 99 (1953).

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[9] T. S. Danowski, J. biol. Chem. *139*, 693 (1941).

[10] E. Flock, J. L. Bollman, F. C. Mann, and E. C. Kendall, J. biol. Chem. *125*, 57 (1938).

[11] R. J. Ellison, W. P. Wilson, and E. B. Weiss, Proc. Soc. exp. Biol. Med. *98*, 128 (1958).

[12] Gift of Abbott Laboratories.

[13] Gift of Sandoz Chemical Works, Inc.

[14] Gift of Eli Lilly and Co.

[15] J. B. Kahn, Jr. and G. H. Acheson, J. Pharmacol. exper. Therap. *115*, 305 (1955).

Table I

Effect of insulin on potassium uptake at 37°C by erythrocytes previously stored at 4°C for 6 days

		No. of experiments	Potassium Uptake (mEq/l of cells ± S.D.) Incubation Time, min		
			15	60	180
Human, Normal	Controls	8		1·4 ± 0·15	3·3 ± 0·86
	Insulin	8		1·4 ± 0·15	3·1 ± 0·74
Human, Diabetic	Controls	1			0·8 ± 0·006
	Insulin	1			0·8 ± 0·006
Chicken, Normal	Controls	6	3·4 ± 0·14	5·2 ± 2·61	
	Insulin	6	3·1 ± 0·33	5·3 ± 2·78	

bated normal human erythrocytes and that this blocking action of ouabain was not modified by insulin.

D'Amico[16] and P. P. Foà

Department of Physiology and Pharmacology, The Chicago Medical School, Chicago 12 (Illinois), December 5, 1958.

Riassunto

La conservazione al freddo degli eritrociti umani e di pollo per 6 giorni causa una fuoruscita di potassio dai globuli. Parte del potassio rientra nelle cellule durante incubazione a 37°C.

La uabaina blocca questo fenomeno. L'insulina non ha effetto significativo nè sull'assunzione di potassio da parte del globulo rosso, nè sull'azione bloccante della uabaina.

[16] Trainee, Diabetes Teaching Grant No. 2A 5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

Glucose and Potassium Transfer in the Isolated Heart of *Venus mercenaria*[1]

The measurement of glucose uptake by the isolated rat diaphragm has been used for the determination of plasma insulin activity, but has several disadvantages. Among these are the difficult dissection, the variability of uptake from sample to sample, the relatively flat slope of the dose-response curve and the high cost. The heart of *Venus mercenaria*, the common sea clam, is a thin walled organ, of remarkably uniform size, cheap and easily obtained. It can be excised with little manipulation and without injury and continues to beat for 1 h or more when immersed in artificial sea water. Purpose of this work was to study the effect of insulin on the uptake of glucose and potassium by the clam heart *in vitro*.

Table I

Effect of insulin on glucose and potassium uptake by clam hearts incubated at 20°C in artificial sea water containing glucose

	No. of experiments	Glucose uptake (mg/g/h ± S.D.)	Potassium uptake (mEq/g/h/ ± S.D.)
Controls . .	10	2·08 ± 0·54	2·39 ± 0·40
Insulin . . .	10	2·15 ± 0·73	2·80 ± 0·58

Clams were obtained from local commercial sources and kept in a wet burlap sack at about 4°C. Live animals, which would tighten their shell shut when gently tapped, were cracked with a hammer. The heart was excised by cutting the large blood vessels, the piece of intestine which passes through it was slipped out gently and the heart was allowed to contract in artificial sea water of Van't Hoff[2], prepared without KCl. After changing the liquid three times to allow complete rinsing of the heart cavities, 2 hearts were placed in a 20 ml beaker with 4 ml of sea water containing KCl (K = 5 mEq/l) and glucose (2 mg/ml). To some beakers glucagon-free insulin[3] (0·5 u/ml) was added. In other experiments the hearts were immersed in sea water containing ouabain[4] ($12{\cdot}5 \times 10^{-4}$ mg/ml) for 1 h, before transfer to the incubation medium. Incubation was carried out in a Dubnoff shaker at 20°C in an atmosphere of 95% O_2–5% CO_2 and moving at the rate of 50 oscillations per min. After 60 min the hearts were removed, blotted on filter paper, and weighed on a torsion balance. Duplicate samples of the incubation medium were analyzed for glucose, according to Nelson[5] and for potassium with a Coleman flame photometer.

[1] Aided by a grant from the Chicago Heart Association.

[2] W. J. V. Osterout, Bot. Gaz. *42*, 127 (1906).

Table II

Effect of insulin on potassium loss in pre-digitalized clam hearts incubated in artificial sea water containing glucose

	No. of experiments	Potassium loss (mEq/g/h/ ± S.D.)
Controls	8	1·48 ± 0·60
Insulin	8	1·52 ± 0·79

Table I shows that the uptake of glucose and potassium by the heart of the sea clam is measurable and relatively uniform and that it is not modified by insulin added to *in vitro*. Table II shows that pre-digitalized hearts lose potassium into the incubation medium and that this loss is not influenced by insulin.

D'Amico[6] and P. P. Foà

Department of Physiology and Pharmacology, The Chicago Medical School, Chicago 12 (Illinois), December 29, 1958.

Riassunto

Il cuore di *Venus mercenaria* consuma glucosio durante incubazione in acqua di mare artificiale. L'assunzione di glucosio è accompagnata da una penetrazione di potassio nell'organo. Cuori pretrattati con uabaina diffondono potassio nel medium di incubazione. L'insulina non modifica né l'assunzione di glucosio e potassio dei cuori normali né la diffusione di potassio dai cuori digitalizzati.

[3] Gift of Eli Lilly & Co.

[4] Gift of Sandoz Chemical Works, Inc.

[5] H. Nelson, J. biol. Chem. *153*, 375 (1944).

[6] Trainee, Diabetes Teaching Grant No. 2A-5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

An Anticonvulsant Effect of Monoamine Oxidase Inhibitors

Chen *et al.*[1] observed that reserpine, though a sedative, lowers the threshold for electroshock and pentylenetetrazol (Metrazol) convulsions in mice. The time course of the effect coincides roughly with the lowering of brain serotonin (5-hydroxytryptamine) and norepinephrine[2]. In contrast, phenothiazine tranquilizers do not lower the brain amines and have no clear-cut influence on electro-

[1] G. Chen, C. R. Ensor, and B. Bohner, Proc. Soc. exp. Biol. Med. *86*, 507 (1954).

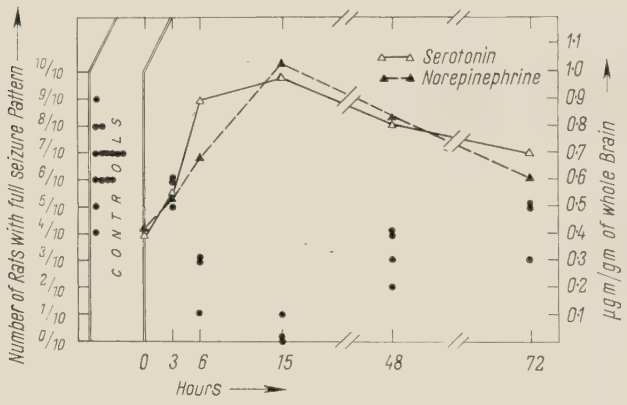
[2] B. B. Brodie, J. S. Olin, R. G. Kuntzman, and P. A. Shore, Science *125*, 1293 (1957).

Effect of various drugs on electroshock seizures

	No. of rats with full seizure pattern	Mean latent period for tonic extension
	per group of 10	seconds
Controls	6·7 ± 1·23 S. D. (15 groups)	4·0 ± 1·19 S. D.
Iproniazid 100 mg/kg	1/10, 0/10, 0/10	—
JB 516 10 mg/kg	0/10, 0/10, 0/10	—
JB 807 50 mg/kg	0/10, 0/10, 0/10	—
Diphenylhydantoin 25 mg/kg	0/10, 0/10, 0/10	—
Reserpine 5 mg/kg	10/10, 10/10, 10/10	1·1 ± 0·05 S. D.
Isoniazid 25 mg/kg	5/10, 7/10	4·3 ± 1·32 S. D.
Isoniazid 100 mg/kg	10/10, 10/10, 7/10	2·5 ± 1·06 S. D.

shock convulsions[3]. Since the brain content of the amines is increased by monoamine oxidase inhibitors[4], it occurred to us that these drugs might antagonize evoked seizures.

Three irreversible monoamine oxidase inhibitors were studied: Iproniazid (1-isonicotinyl-2-isopropylhydrazine), JB 516 ([phenylisopropyl]-hydrazine), and JB 807 (1-[phenylisopropyl]-2-isopropylhydrazine). Rats received the drugs intraperitoneally and seizures were induced by supramaximal electroshock[5]. An anticonvulsant effect was defined as suppression of tonic extension of the hind legs, a criterion widely used in screening for antiepileptic drugs.



Effect of iproniazid (100 mg/kg) on brain levels of serotonin and norepinephrine and on electroshock seizures in rats. The drug was injected intraperitoneally into female Sprague-Dawley rats weighing 125–150 g. At various times thereafter, a 60 cycle AC current of 300 mA was applied through corneal electrodes for 0·3 s (2 to 3 times maximal shock). Each solid circle represents the number of animals per group of 10 showing tonic extension of the hind limbs. No animal was shocked more than once. Amine levels in whole brain were measured by previously described methods [6]. Each triangle represents the average value of six animals.

Iproniazid (100 mg/kg) suppressed the tonic extensor phase and raised levels of brain amine two to threefold. Both effects were maximal in 15 h and persisted for about two days (Fig.). JB 516 (10 mg/kg), a more potent and faster acting inhibitor[7], in 6 h also prevented the tonic extensor phase and produced a similar rise in brain amines. The anticonvulsant effect and elevation of brain amines gradually declined over two days. JB 516 had a cumulative action; for example, a single dose of 5 mg/kg showed little activity, but given daily for seven days exerted a pronounced effect. JB 807 (50 mg/kg) after 6 h was also anticonvulsant (Table) and had raised the brain amines. Isoniazid (isonicotinylhydrazine), a congener of iproniazid, did not elevate brain amines[4] and had no anticonvulsant effect 2 or 15 h after doses of 25 mg/kg. In doses of 100 mg/kg, the drug facilitated electroshock seizures, presumably due to its anti-pyridoxal action[8].

In contrast to the monoamine oxidase inhibitors, reserpine (5 mg/kg) in 2 h increased the response to electroshock and shortened the latent period for appearance of tonic extension (Table).

The converse effects of reserpine and monoamine oxidase inhibitors on electroshock convulsions suggest that their actions were not due to the drugs *per se* but were related to the opposite changes induced in brain amines. Further support for this view was obtained from experiments in which the combination of reserpine and iproniazid elicited opposite effects, depending on which drug was given first[9]. Rats were treated with reserpine 2 h before iproniazid. 2 h after the iproniazid administration the brain amines were depleted, and an enhanced response to electroshock typical of reserpine was noted. In other experiments animals were given reserpine 2 h after iproniazid. 2 h following the reserpine administration, an anticonvulsant action typical of iproniazid was observed and the brain amines showed little change from normal since they had been liberated when monoamine oxidase activity was blocked. Not only are these results in accordance with the view that reserpine and iproniazid do not act *per se* but they suggest that the level of free brain amines may be an important factor in the effects on electroshock.

Diphenylhydantoin (Dilantin) in doses of 100 mg/kg twice daily for four days has been reported to elevate brain serotonin levels in rats[10]. We observed that doses of 25–75 mg/kg blocked the tonic extension component of electroshock, but did not increase the brain amines. The anticonvulsant action of diphenylhydantoin in rats

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[8] R. H. Rielly, K. F. Killiam, E. H. Jenny, W. H. Marshall, T. Tausig, and N. S. Apter, J. Amer. med. Assoc. *152*, 1317 (1953).

[9] P. A. Shore and B. B. Brodie, Proc. Soc. exp. Biol. Med. *94*, 433 (1957).

[10] D. D. Bonnycastle, N. J. Giarman, and M. K. Paasonen, Brit. J. Pharmacol. *12*, 228 (1957).

does not appear, therefore, to be associated with a measurable rise in brain amine levels.

The data presented here show that monoamine oxidase inhibitors elevate norepinephrine and serotonin brain levels and suppress the tonic extensor component of electroshock seizures in rats. In contrast, reserpine releases brain amines and facilitates electroshock seizures. The results do not permit the conclusion that the effects of the drugs are attributable to changes in serotonin or norepinephrine, but suggest the possibility that a substance released by reserpine and metabolized by monoamine oxidase is involved[11].

Further studies have demonstrated that JB 516 and JB 807 suppress the tonic extensor phase of convulsions evoked by intravenously administered pentylenetetrazol in mice. A detailed account of these results will be published elsenwhere.

The possible clinical usefulness of monoamine oxidase inhibitors in epilepsy is now under investigation.

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Laboratory of Chemical Pharmacology, National Heart Institute, U. S. Department of Health, Education, and Welfare, Bethesda (Maryland), December 11, 1958.

Zusammenfassung

Inhibitoren der Monoaminoxydase haben eine antikonvulsive Wirkung, die mit einer Erhöhung des Serotonin- und Noradrenalingehaltes im Gehirn verbunden ist. Da anderseits Reserpin durch Freisetzung zu einer Verminderung dieser Amine im Gehirn führt und die Krämpfe verstärkt, ist es wahrscheinlich, dass gewisse physiologisch aktive Amine bei der Entstehung experimenteller Konvulsionen eine entscheidende Rolle spielen. Die Resultate stützen die Annahme, dass gewisse Formen von Epilepsie auf eine lokale Störung im Stoffwechsel der Gehirnamine zurückzuführen sind.

[11] In preliminary experiments, harmaline, a reversibly acting monoamine oxidase inhibitor [12] was found to raise the brain levels of amines and block the tonic extensor phase of electroshock seizures in rats.

[12] S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, Biochem. Pharmacol., in press.

* Fellow, Life Insurance Medical Research Fund.

Regeneration of Cartilaginous Matrix from the Dissociated Chondrocytes *in vitro*

Recently, it has been well established that the constituent cells of a precartilaginous blastema can, after dissociation, reaggregate into a chaotic assemblage and finally differentiate into organised cartilage[1]. However, if the dissociation of the cells is done in the stages before the onset of visible differentiation of cartilage, the second process, that is, the transformation of the initial reaggregates into organised cartilage, can be considered as essentially the same process as the differentiation of cartilage from the intact precartilage[2]. This provides, therefore, little direct information as to the relative importance of the intercellular matrix and the constituent cells in the maintenance of the tissue architecture. It seemed to the present author that useful information on this point might be obtained by studying the process of tissue reconstitution from differentiated chondrocytes after freeing them from the already deposited matrix. In this paper, the results of an experiment along these lines will be described.

Experimental. Suspensions of chondrocytes were made from femora and tibiae of chick embryos after 8 days' incubation. In this material, a considerable amount of cartilaginous matrix, which gives strong metachromatic staining with toluidine blue, is deposited intercellularly. The demarcation of diaphysial and epiphysial zones is quite clear in femora, and hypertrophy of chondrocytes is conspicuous.

The procedure of making cell suspension by trypsin digestion was essentially the same as that established by Moscona[3] except for slight modification[2]. With the present material, however, much more prolonged treatment by trypsin than that applied to the precartilaginous material was necessary, in order to obtain a soft mass which could then be pipetted with a small-bore pipette or syringe to make a cell suspension. Approximately 30 min pretreatment in Ca- and Mg-free saline, and a further one hour's incubation in the trypsin solution, proved adequate. The periosteum, which was developed as a thin conical cylinder around the diaphysis of the femur, was so resistant to enzymatic digestion that it remained as a hard sheath, even after such prolonged treatment. The final suspension did not, therefore, contain any cells of this tissue.

The description of the sequence of events which will be given below is based on 34 specimens fixed in various steps of the experiment, together with some smeared preparations of the discrete cells of the suspension. For the histological examination, alternative sections were made, half being stained with haematoxyline (Meyer or Heidenhain) and the other half stained with toluidine blue for the detection of cartilaginous matrix.

The observation of the smeared preparations of the discrete chondrocytes showed only the simple spherical cells which are commonly found in cell suspensions of any kind of embryonic tissue, without showing any structural characteristics of the initial chondrocytes, i. e. hypertrophic and flattened cells (Fig. A). Mortality of cells in the suspension was much higher than in suspensions made from earlier embryonic stages. A higher mortality of discrete cells obtained from older tissue was also described for nervous tissue[4]. The fatal effect of prolonged trypsin treatment and trauma by vigorous pipetting are likely to be the reasons for the high percentages of damaged cells.

Reaggregation of the discrete cells was tried in two ways: (1) The cultivation of the very slimy soft mass obtained after centrigugation of the suspension, or (2) the cultivation of a dense suspension within a hollow ground slide or solid watch-glass filled with the liquid medium. A mixture of modified Tyrode[5], horse serum, and embryo extract in equal proportions served as the culture medium.

During 24 hours' cultivation within the liquid medium, the isolated cells assembled spontaneously into small reaggregates, whilst the slimy aggregates formed by centrifuging lost their stickness and became more or less firm, probably owing to the contraction of a very viscous substance which surrounds the intact cells and appears first after centrifuging. These initial aggregated masses

[1] A. Moscona and H. Moscona, J. Anat. *86*, 287 (1952). – P. Weiss and A. Moscona, J. Embryol. exp. Morph. *6*, 238 (1958).

[2] T. S. Okada, Exp. Cell Res. *16*, 437 (1959).

[3] A. Moscona, Exp. Cell Res. *3*, 535 (1952).

[4] M. Cavanaugh, Exp. Cell Res. *9*, 42 (1955).

[5] M. Jones and S. L. Bonting, Exp. Cell Res. *10*, 631 (1956).

were colonies of loosely packed spherical precartilage-like cells; the intercellular space gave no metachromatic staining with toluidine blue (Fig. *B*). Neither hypertrophied nor flattened cells were found in this stage. The periphery of such reaggregate was already surrounded by one or two layers of flattened mesenchymal cells and was reminiscent of perichondral organisation.

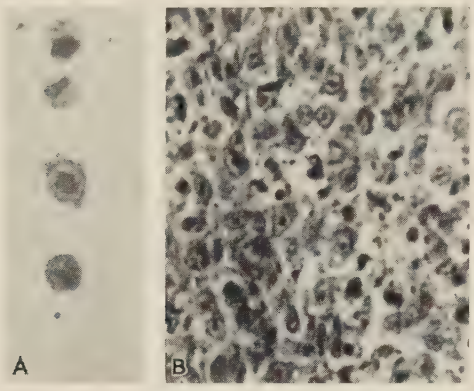

Fig. A.—Smear of a cell suspension of the chondrocytes from 8-day embryo (Meyer Haematoxyline)

Fig. B.—Initial reaggregate of the dissociated chondrocytes, showing the assemblage of spherical cells (Heidenhain Haematoxyline)

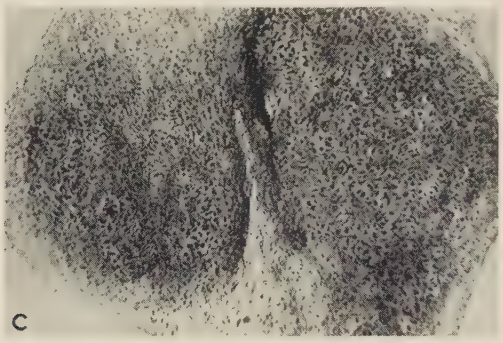

Fig. C.—Regeneration of matrix after 2 days' culturing on lens paper (Toluidine blue)

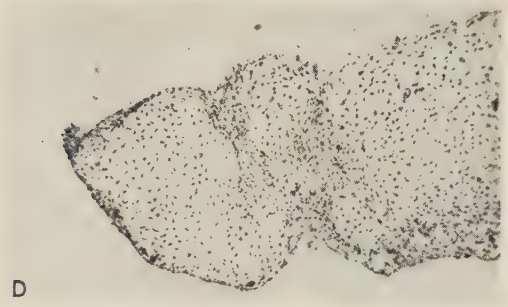

Fig. D.—Colony of cartilaginous nodules formed after 3·5 days' culturing on lens paper (Heidenhain Haematoxyline)

After further cultivation of the reaggregates in fesh liquid medium for 24 hours, several small foci of chondrification began to appear; here the intercellular space gave faint metachromatic staining with toluidine blue. At this stage the reaggregates were transferred to lens papers placed over the liquid medium[6]. In this culture condition chondrification proceeded quite well. Sections of the cultures fixed after 36 hours' cultivation upon lens paper showed the production of conspicuous amounts of metachromatic substance within the intercellular spaces (Fig. *C*).

Cultures on lens paper lasted for 3·5 days without changing medium. At this terminal stage, all cultures were fixed, and on examination were found to be colonies of cartilaginous nodules surrounded by a sheath of flattened mesenchymal cells, which now can be safely called perichondrium (Fig. *D*). All chondrocytes had regained their original structural characteristics.

Discussion. The results described above demonstrate that the isolated chondrocytes of fully differentiated cartilage, which were obtained after complete trypsin digestion of the intercellular matrix, are able to reaggregate and to re-establish the original histological architecture of cartilage by regenerating the intercellular matrix. It is noteworthy that this process of the matrix regeneration is always accompanied by some structural changes in the constituent cells; the simple spherical form which chondorcytes assume in the initial reaggregates, changes and is replaced by the original cellular structure. The loss of the structural characteristics of chondrocytes, accompanied by the disappearance of the intercellular matrix, has been recorded in the culture of cartilaginous fragments in a medium containing excess amounts of Vitamin A[7]. In these cases, however, both the recovery of synthetic activity in regeneration of matrix and the return of original cellular structure were reported as very incomplete, after transferring into normal medium[8], whereas in the discrete chondrocytes here described, the recovery was found to be nearly complete both of their morphology and of their function of secreting the intercellular matrix. Therefore, the altered chondrocytes appearing in these two different experiments must be considered as strikingly different from each other in their capacity for differentiation.

In the sequence of the events described in this paper, no metachromatic substance was found in the intercellular regions of the cell assemblages fixed soon after reaggregation of the dissociated cells. This fact suggests that the contiguity and stability of the chondrocytes in the initial reaggregate may not be maintained by the same cementing substance as that filling up the intercellular spaces of the original cartilage.

Since the metachromatic matrix always appeared in aggregates fixed at a later time in the course of the experiments, one has the impression that mutual assembly of the discrete cells may be the prerequisite condition for the recovery of their synthetic activity. At present, nothing definite can be said on this point, as we do not know yet whether or not the dissociated chondrocytes, had they been cultured in such exprimental conditions as to prevent their reaggregation, could regain their cellular activity of secreting the matrix substance as such. One of the specimens which was fixed at 24 h after culturing the centrifuged slimy mass may, however, have some bearing on this point. In this reaggregate so many damaged cells were incorporated, that healthy cells were singly dispersed among them, except for one locus, where 10–15 healthy cells were assembled, and it was just in this region that chondrification was observed to have began. This observation, although far from conclusive, seems to suggest that there is a limiting minimum mass, below which the regeneration of matrix and re-establishment of cartilaginous tissue architecture will not occur[9].

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[9] A. Moscona, Proc. Soc. exp. Biol. Med. *92*, 410 (1956).

Acknowledgement. The author is very much indebted to Prof. C. H. Waddington for encouragement and for the interest he has shown in this work; and to Mrs. R. M. Clayton for reading the manuscript. The author has been the holder of Macauley Fellowship from Edinburgh University, which has made his work in the U. K. possible.

T. S. Okada

Institute of Animal Genetics, University of Edinburgh (on leave of absence from the Zoological Institute, Faculty of Science, University of Kyoto, Japan), December 12, 1958.

Zusammenfassung

Aus 8 Tage alten Hühnerembryonen wurden durch Verdauung der intrazellulären Matrix des differenzierten Knorpelgewebes dissoziierte Chondrozyten gewonnen. Diese Chondrozyten schlossen sich wieder zu Kolonien lose gepackter sphärischer Zellen zusammen. Durch Regeneration der interzellulären Matrix bildete sich in den Organkulturen solcher Reaggregate die ursprüngliche Knorpel-Architektur wieder aus.

Normal Count and Physiological Variability of Rabbit Blood Basophils[1]

Rabbit blood contains an exceptionally great number of basophil granulocytes. Basophil countings from rabbit blood smears have been performed by various authors, yielding highly varying results. Using a direct chamber counting method[2], the present study of the basophil count in rabbit blood under physiological variations was undertaken.

Methods. 159 six-months-old white rabbits of both sexes and of known homogeneous breed were used. They were separately caged, at a temperature of 20° ± 1°C, fed on oats, hay, and sugar beets. The animals were carefully handled and trained to accommodate in blood-sampling boxes. For blood sampling, the ear was tapped lightly by finger to stimulate blood circulation, and a prick with a 14-gauge needle or a small incision in one of the ear veins usually secured a free flow of blood drops. After discarding the first drop, 20 µl of blood was drawn into a standard blood pipette, and quickly transferred into a glass tube containing 180 µl of a modified Moore & James toluidine blue solution[3]. The tube was shaken until complete hemolysis of the red cells. A solution of 0·05% toluidine blue in 50% watery propylene glycol was used in few cases. The diluted blood was then transferred by capillarity to Fuchs-Rosenthal counting chambers (0·2 mm deep) and the cells allowed to settle. Counting slides were kept in a covered Petri dish floored with a damp filter paper to avoid evaporation. Two chambers of 16 mm² area each were counted for basophils, and two big squares of 1 mm² area each for obtaining total leucocyte counts. A binocular microscope with × 10 oculars and a × 10 achromatic plane objective was used; a blue filter contrasted the metachromatically stained basophils against the faintly blue-stained leucocytes of different types.

Results. Figure 1 presents distribution curves, as well as mean values ± standard error, standard deviations and range of 9 a.m. basophil counts (absolute and differential) of the first blood samples ever taken from all rabbits under study. The curves are more or less positively skewed[4].

A very slight variation in the morning count from day to day was encountered (Table). A study of diurnal variation in the number of basophils in 58 male rabbits, however, revealed a statistically significant ($P < 0{\cdot}001$) increase in late afternoon (3 p.m.) as compared to the comparatively low morning (9 a.m.) count (Fig. 2).

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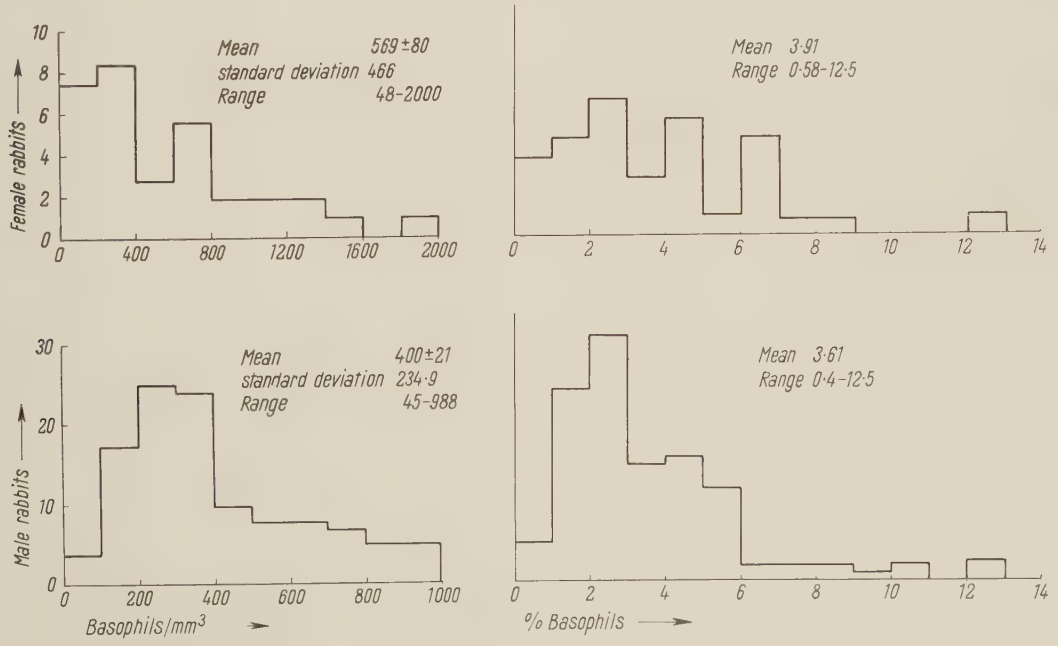

Fig. 1.—Distribution curves of first absolute and relative basophil leucocyte morning (9 a.m.) counts ever taken from 116 male and 43 female adult rabbits. Mean ± standard error, standard deviation, and range are indicated.

The average of 1142 basophil counts at different times of day was 516·4 ± 10·8/µl blood (st. dev. ± 366). The mean of 426 basophil counts of females was 545·6 ± 19·3 (st. dev. ± 399), which is insignificantly higher than the mean of 716 basophil counts of male rabbits, showing 499 ± 12·9 (st. dev. ± 344).

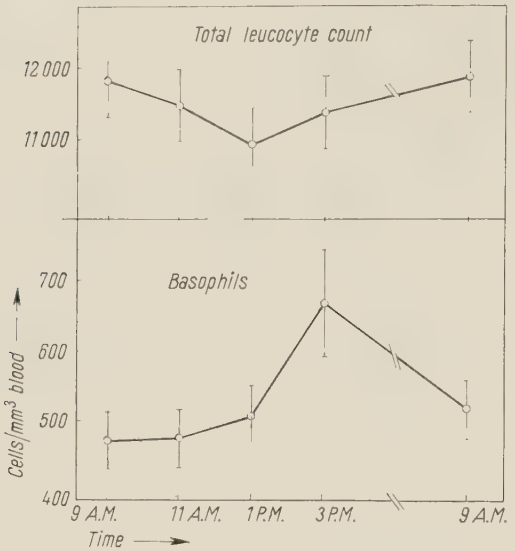

Fig. 2.—Diurnal variation of blood basophil and total leucocyte counts for 58 male adult rabbits ($\frac{\delta}{1}$: mean ± standard error).

During pregnancy, 6 females demonstrated a clear tendency towards a fall in basophil count, most pronounced on the day of delivery and the third day after delivery (Fig. 3).

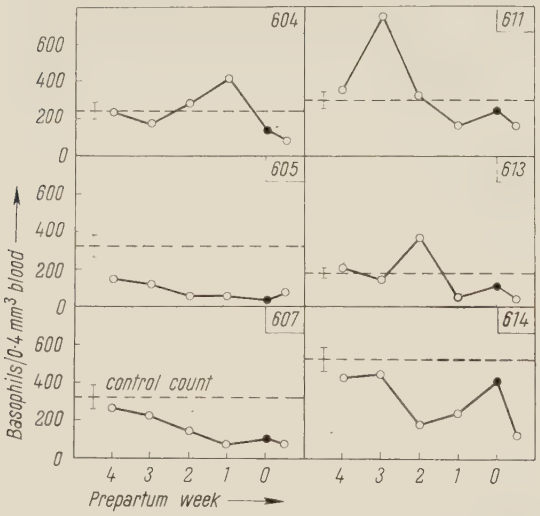

Fig. 3.—Blood basophil count variations for 6 rabbits during pregnancy (o: counts before or after delivery, •: count on day of delivery; – – –: mean of 4 control counts taken on 4 successive days before copulation with standard error indicated by vertical line).

Discussion. By indirect counting methods, the number of basophils in rabbit blood ranged from 0–950 cells/µl blood, with differential counts of 0–12%[5]. Direct counts of the present study, however, revealed a minimum count of 45 basophils/µl blood, corresponding to a differential count of 0·4%. Although a wide individual variation in the counts was observed, they differed but slightly from day to day under standard conditions. The higher level of basophils in the female group and the afternoon rise in both counts are in accordance with reported findings in normal humans[6]. Even the decrease observed during pregnancy agrees with the decreasing tendency reported in pregnant women. An influence of adrenocortical as well as female sex hormones on the number of circulating basophils has been suggested previously[7], and may also be responsible for these variations.

Variations in the morning basophil count in rabbit blood (counts per mm³ blood, at 9 a.m.) on successive days.

Day	Males			Females		
	No. of counts	Mean ± standard error	Standard deviation ±	No. of counts	Mean ± standard error	Standard deviation ±
1	128	475 ± 23·6	267	43	552 ± 72·0	472
2	128	528 ± 20·5	232	43	453 ± 49·9	327
3	70	422 ± 34·0	284	43	524 ± 46·1	302
4	58	428 ± 35·8	272			
5	50	422 ± 45·0	318			
Total	434	469 ± 12·9	269	129	509 ± 32·9	374

A.-W. A. Boseila[8]

Connective Tissue Research Laboratory, University Institute of Medical Anatomy, Copenhagen (Denmark), January 19, 1959.

Zusammenfassung

Direkte Zählung von basophilen Leukozyten 195 männlicher und weiblicher Kaninchen in einer Blutkammer ergab eine Durchschnittszahl von 516 pro mm³ Blut. Eine signifikante Zunahme von Basophilen findet nachmittags statt. Während der Schwangerschaft wurde eine Abnahme der Basophilen beobachtet.

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The Embryonic Origin of the Intrinsic Limb Musculature in Amphibia, Salientia

The mesodermal cells that develop into the intrinsic limb muscles, in tetrapods, are generally considered to be exclusively somatopleural in origin[1–6]. Milaire[7] has re-

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cently contested this interpretation for reptiles. In view of MILAIRE's results and the earlier report of FIELD[8] (later denied by BYRNES[1]) that myotomes contribute to the limb musculature in frogs, it seemed pertinent to re-examine the problem in the latter group.

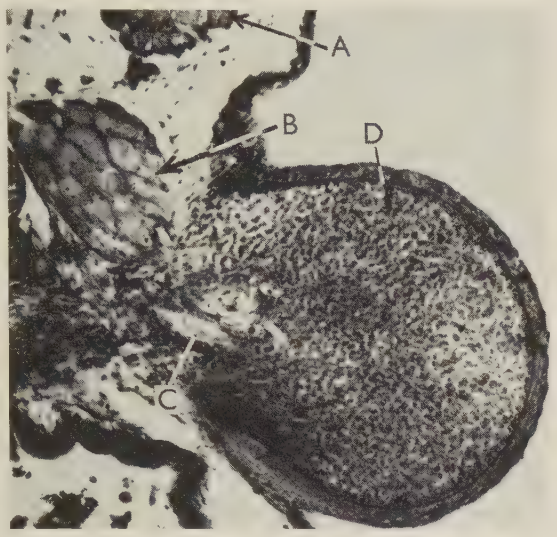

Fig. 1.—Transverse section through the hind limb bud of *Rana ridibunda*. *A* = myotome; *B* = residuum of primary muscle bud; *C* = secondary muscle bud; *D* = limb bud

Normal series. Normal limb bud development was followed in *Bufo calamita*, *Megophrys pelodytoides*, and *Rana ridibunda*. A closely spaced series extending from limb bud stages I–IX (TAYLOR and KOLLROSS[9]) was employed for each genus. The primary limb protuberance forms by the accumulation of mesenchyme cells which leave the coelomic epithelium and migrate outwards to establish contact with the adjacent thickened epidermis. Subsequent growth of the fore-limb (except for nerves and blood vessels) is entirely by the *in situ* proliferation of these parietopleural cells. In the hind limb region, however (at about stage IV), a bud separates from the medioventral edge of the 8th trunk myotome. This extends towards the hind limb 'anlage', and then itself constricts a much smaller secondary bud, which grows into and supplements the mesenchyme of the limb (Fig. 1). The residuum of the primary bud makes no contribution to the future limb, but bends ventrally to contribute to the abdominal musculature.

These results were tested by two experimental techniques. For each experiment 25 specimens of *Bufo calamita* and of *Rana ridibunda* were employed.

Transplantation experiments. Early fore and hind limb primordia (stage III–IV) were implanted in the abdominal wall of the same individual. At the end of metamorphosis the explanted and normal limbs were compared topographically and histologically. Fore limb explants differed from the controls only in their slightly slower growth rate and in a variable degree of distortion of their skeletal elements. Hind limb grafts, however, consistently showed a marked paucity of thigh musculature (Fig. 2), although the distal musculature of the same limb and its skeleton as a whole were quite normal.

Marking experiments. Stages just prior to the formation of the limb bud protuberance were employed. Fine carbon particles were pressed into the ventral and ventro-medial borders of trunk myotomes 7–9. The larvae were killed at the late paddle stage, fixed in 70% ethanol and cleared in *N*/10 potash solution. Particle recoveries were made from the tissues of the abdominal wall only, in 39 individuals, and from the proximal segments of the hind limb as well as the abdominal wall in 4 others. In the remaining 7 specimens, no particles at all were located.

Discussion. HARRISON[10] showed, in Anura, that the nerve elements which normally innervate the muscles of hind limbs play no part in the morphogenesis of those muscles. The transplantation results, therefore, cannot be attributed to inadequate innervation. In any event, such an explanation could not account for the normal muscle condition in the distal segments of the same limb. The results are interpreted, therefore, as indicating that the dual origin of the hind limb mesoderm, observed in the normal series, has a real developmental significance, and that a primordium deprived of its myotomic component cannot adequately rectify this deficiency by compensatory proliferation of the somatopleural factor. This accords with recent experimental work[11,12] which indicates that the development of the anuran limb is of the mosaic type.

Failure to trace carbon particles from somites to limbs forms one of the main arguments for a purely somatopleural interpretation of limb muscles[6,13,14]. But marking the ground tissue of a localized area does not preclude the possibility of cells migrating past the labelled zone, particularly if they arise by secondary constriction from within the main mass. In such cases it is suggested that the carbon method should be employed only in a confirmatory role, and significance attached only to positive results. Interpreted in this way, the few positive results gained during this investigation provide at least some support for the conclusions based on normal development.

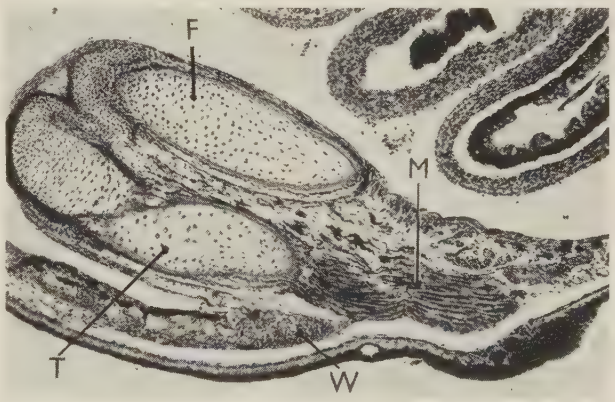

Fig. 2.—Transverse section through the abdominal wall and implanted limb bud of *Rana ridibunda*. *F* = femur; *M* = tibio-fibular musculature; *T* = tibio-fibula; *W* = abdominal wall musculature

Regarding the purely somatopleural nature of the fore limb mesenchyme as compared with the situation in the hind limb of frogs, it is noteworthy that HARRISON[15] described a comparable condition in the salmon. He showed that the pectoral fin originates exclusively from the somatopleure whilst most of the other

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fins receive direct myotomic contributions. He demonstrated, also, that the posterior half of the dorsal fin derives buds from the myotomes, but that the anterior half of the same fin receives no myotomic tissue whatsoever. The possibility of expressing the myotomic role, in limb and fin formation, in terms of a cephalocaudal gradient, therefore, should not be excluded.

I. Griffiths

Department of Zoology, Birkbeck College, University of London, October 31, 1958.

Zusammenfassung

Das Blastem, welches sich zur freien, hinteren Gliedmassenmuskulatur entwickelt, stammt bei Anuren von parietalem Mesoderm und von Ursegmenten ab. Das Vorderbein-Mesoderm dagegen ist ausschliesslich parietal. Diese Feststellungen werden durch Befunde an transplantierten Beinknospen und durch Markierungsversuche mit Kohlepartikeln gestützt.

Die Brauchbarkeit des Kohlemarkierungs-Verfahrens wurde speziell in ihrer Anwendung auf die Feststellung der Wanderrouten von Muskelzellen geprüft.

p-Aminohippuric Acid Accumulation in Kidney Slices in Cloudy Swelling

The clearance of *p*-aminohippuric acid (PAH) is generally considered as one of most important tests of kidney tubular function. Injected PAH is brought with the blood to the tubule cells, and then passes into the cells and is finally excreted in the urine. The peculiar mechanism of this phenomenon is, however, not clear.

Cross and Taggart[1] have recently shown that PAH is accumulated within kidney slices respiring *in vitro* when these are incubated in the presence of this substance. The same authors have also shown that 2,4-dinitrophenol (DNP), as well as some other related substances which inhibit the oxidative phosphorylation, prevents this accumulation. Other substances which decrease the respiration rate such as cyanide, azide, arsenite, fluoride, iodoacetate, fluoroacetate, and mercuric hydrochloride also decrease the accumulation of PAH within the slices. Acetate, on the other hand, has a powerful stimulating activity. It thus seems very probable that oxidative phosphorylation is involved in the accumulation phenomenon.

Oxidative phosphorylation is decreased in tissues showing cloudy swelling [2,3]. It therefore seemed interesting to study the influence on the accumulation of PAH within kidney slices *in vitro* of treatments capable of producing cloudy swelling in this organ. The results of such an investigation are described in this paper.

Several types of damaging treatments were used: (1) Intraperitoneal injection in the rat of the toxin of *Salmonella typhi murium* (0·5 ml of a 24 h culture in broth, killed by heating at 70°C for 1 h). The animals were killed 24 h after the injection. This type of treatment has been found able to produce a typical cloudy swelling in rat kidney[4,5]. ATP concentration of the organs of treated animals is decreased[6]. (2) Intraperitoneal injection of the α-toxin of *Staphylococcus pyogenes aureus* (10 hemolytic combination dosis; the toxin was kindly supplied by the Istituto Sieroterapico Vaccinogeno Toscano, Siena). This toxin produces a diffused cloudy swelling in rat kidneys, as well as a strong decrease of P/O ratio[7]. The histological changes are still present 24 h after the injection, but maximum alterations were found 96 and 120 h after the injection. (3) Intraperitoneal injection of DNP (3 mg/100 g body weight). The rats were killed 24 h after the injection. This treatment was found to produce cloudy swelling in several rat organs[8,9], as well as decrease of P/O ratio[8]. (4) Intraperitoneal injection of thyroxine (Hoffmann-La Roche, 1 mg/100 g body weight, for 2 days). The rats were killed 24 h after the last injection. Thyroxine produces decrease of P/O ratio in liver mitochondria[10–12] as well as cloudy swelling[13].

Albino rats weighing 250–300 g were used. They were killed by bleeding. Their right kidney was immediately taken out and weighed. A small fragment was used for nitrogen determination (method of Kjeldhal). Another fragment was used for the preparation of histological specimens. The remaining portion of kidney cortex was used to prepare hand cut slices for the study of PAH accumulation. This was made by the method described by Cross and Taggart[1], using Warburg manometers at 25°C, with 90–100 mg of slices (wet weight) and 0·001 *M* sodium *p*-aminohippurate (S.I.M.E.S., Milano). Oxygen was the gaseous phase. When added, sodium acetate had the final concentration 0·01 *M*. Oxygen uptake was recorded for 1 h. After this time, the flasks were removed and immediately transferred to the cold room at 2°C. The slices were collected on a metal nest filter and then homogenized with a gum pestle. PAH concentration in both slices and suspension fluid was determined according to the procedure of Cross and Taggart[1]. Readings of the optical density were made at 450 mμ in a Beckman Mod. DU spectrophotometer. The following data were calculated in each experiment: (1) QO_2: this was obtained by dividing the microliters of O_2 consumed in 1 h by the mg (wet weight) of the used amount of tissue. (2) the S/M ratio between the micromoles of PAH found within the slices (S) per g and those remaining in the suspension medium (M) per ml. (3) the quotient between this value and QO_2 (S/M: QO_2). This is indicated in the Table as Q. The standard deviation was calculated for each average. The significance of the differences between two averages was estimated by calculating the '*t*' value of Fisher. The Table reports the results. It is clear from the Table that QO_2 is significantly increased after all types of treatments. The increase was, however, particularly high after the injection of thyroxine. The significance of the results is less when the values are referred to the nitrogen content, but remain particularly high for the rats treated with thyroxine or with DNP. In fact, in last cases, nitro-

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Respiration and PAH accumulation within kidney slices of animals with cloudy swelling

Type of treatment	None	*S. typhi murium* toxin	*Staphylococcus* toxin (24 h)	*Staphylococcus* toxin (96 h)	*Staphylococcus* toxin (120 h)	DNP	Thyroxine
No. of experiments	18	7	9	4	4	6	7
Nitrogen (mg/g) . .	27·1 ± 1·20	28·6 ± 0·50	28·2 ± 1·00	31·3 ± 1·40	28·4 ± 0·80	27·3 ± 0·20	27·7 ± 0·80
QO_2	1·06 ± 0·11	1·33 ± 0·07	1·34 ± 0·14	1·34 ± 0·04	1·23 ± 0·10	1·37 ± 0·08	1·51 ± 0·11
QO_2 acetate	1·41 ± 0·11	1·63 ± 0·22	1·64 ± 0·14	1·49 ± 0·16	1·62 ± 0·10	1·62 ± 0·18	1·68 ± 0·30
Stimulation % by acetate	33·0 ± 12·0	22·5 ± 17.7	22·3 ± 7·70	11·2 ± 9·30	31·7 ± 1·50	18·2 ± 7·6	11·2 ± 13.0
S/M	5·40 ± 0·63	4·67 ± 1·03	4·79 ± 1.10	4·23 ± 0·75	3·66 ± 1·0	3·33 ± 0·45	3·59 ± 1·09
S/M acetate	6·70 ± 0·80	4·82 ± 0·99	5·43 ± 1·24	5·04 ± 0·76	4·37 ± 1·11	3·24 ± 0·46	4·24 ± 1·35
Stimulation % by acetate	24·0 ± 9·4	3·20 ± 3·00	13·3 ± 11·4	19·1 ± 6·00	19·4 ± 4·60	–2·7 ± 0·50	18·1 ± 12·1
Q	5·21 ± 0·69	3·53 ± 0·88	3·61 ± 0·85	3·13 ± 0·55	3·55 ± 0·12	2·41 ± 0·20	2·40 ± 0·72
Q acetate	4·86 ± 0·80	3·05 ± 0·98	3·52 ± 0·75	3·99 ± 0·40	3·79 ± 0·15	1·98 ± 0·16	2·64 ± 1·07

gen was not significantly increased, while it increased remarkably in all other cases studied. Acetate stimulated in all cases the O_2 uptake. Percent stimulation by acetate was not significantly modified in rats treated with the toxins of *S. typhi murium* or of *S. pyogenes aureus* (after 24 and after 120 h), but was consistently decreased in those treated either with DNP or with thyroxine, or also injected with the Staphylococcus toxin 96 h before.

The S/M ratio, either in the absence or in the presence of acetate, was not significantly decreased in rats treated with *S. typhi murium* toxin and also in those injected 24 h before with the *Staphylococcus* toxin. It was, however, strongly decreased after all other types of treatments used. Stimulation of the accumulation of PAH produced by acetate was consistently decreased in rats treated with the toxin of *S. typhi murium* and in those injected with *Staphylococcus* toxin 24 h before; it was abolished in those treated with DNP, but remained practically unmodified in the other cases.

The values of the quotient S/M: QO_2(Q) were strongly decreased in all groups of rats, either in the presence or in the absence of acetate. The extent of the decrease was particularly high in rats treated with DNP and in those injected with thyroxine.

The histological feature of the kidney of all groups studied was that of cloudy swelling. The rats treated with the *Staphylococcus* toxin sometimes also showed glomerular damage. This consisted of hypernucleosis, dilatation of the glomerular capillaries, and oedema. These alterations were particularly severe in the animals treated 96 h before with the toxin, but were generally decreased 120 h after the injection. Small foci of tubular necrosis were sometimes seen.

These results show that treatments which produce a decrease in the efficiency of oxidative phosphorylation, as well as cloudy swelling in the kidney, produce also a decrease of PAH accumulation within the kidney cortex slices.

The fact that the Q ratios are strongly decreased in all treated groups shows that the increase of the oxidative rate is without effect with respect to PAH accumulation. These facts agree with the hypothesis that the accumulation of PAH within the kidney slices probably depends upon the functioning of oxidative phosphorylation.

B. Fida, G. C. Biaggini, and M. U. Dianzani

Institute of General Pathology of the University of Genoa (Italy), November 26, 1958.

Riassunto

L'incorporazione dell'acido *p*-aminoippurico nelle sezioni di rene di ratto è diminuita nel rigonfiamento torbido provocato dal trattamento parenterale con tossina stafilococcioa, con dinitrofenolo o con tiroxina. Tutti questi trattamenti provocano anche dissociazione delle fosforilazioni dalle ossidazioni.

Nebennierenfunktion und Veränderungen der Leber nach Röntgenbestrahlung

Nach Ganzbestrahlung diverser Säugetiere wurden Störungen verschiedener Leberfunktionen, Leberschädigung in Form der fettigen Degeneration und Gewichtszunahme des gesamten Leberfettes beobachtet[1–3]. Nach Ellinger[1], Betz[2] und Bacq[4] werden diese Veränderungen durch die Dysfunktion des Hypophyse-Nebennieren-Systems bewirkt, als Folge der Belastung des Organismus durch Röntgenstrahlen. Adrenalektomie[2] wie auch Verabreichung von Desoxycorticosteron[1] wirken hemmend auf diese Erscheinungen. Die vorliegende Arbeit beschäftigt sich eingehender mit diesem Problem.

In unseren Versuchen wurden weisse Mäuse beiderlei Geschlechts aus dem H-Stamm verwendet. Sie wurden mit Larsen-Diät und Trinkwasser *ad libitum* gefüttert; in

[1] F. Ellinger, Proc. Soc. exp. Biol. Med. N. Y. *64*, 31 (1947).

[2] E. H. Betz, *Contribution à l'étude du syndrome endocrinien provoqué par l'irridiation totale de l'organisme* (Paris 1955).

[3] M. Skalka (in Vorbereitung).

[4] Z. M. Bacq und P. Alexander, *Fundamentals of Radiobiology* (London 1955).

Tabelle I
Leberfettgehalt bei bestrahlten Mäusen (Weibchen), Operation vor der Bestrahlung

Unbestrahlte Kontrolltiere	3,1 ± 0,57 (8)*
Bestrahlte Tiere mit 2 Nebennieren. . .	7,2 ± 1,71 (12)
Bestrahlte Tiere mit 1 Nebenniere . . .	8,7 ± 1,23 (12)
Bestrahlte Tiere mit $^1/_2$ Nebenniere . .	7,6 ± 1,22 (10)
Bestrahlte Tiere ohne Nebennieren . . .	3,8 ± 1,45 (10)

Tabelle IV
Leberfettgehalt bei bestrahlten Mäusen (Männchen), mit Desoxycorticosteronacetat behandelt

	Ohne Behandlung	Mit Behandlung
Unbestrahlte Tiere . .	3,7 ± 0,60 (6)	3,6 ± 0,84 (6)
Bestrahlte Tiere	7,6 ± 2,12 (10)	7,2 ± 2,06 (10)

den Versuchen mit Adrenalektomie wurde das Trinkwasser durch eine physiologische Lösung ersetzt. Die folgenden Operationen wurden in leichter Äthernarkose durchgeführt: Entfernung beider Nebennieren, Entfernung einer (linken) Nebenniere, Entfernung einer ganzen und einer halben Nebenniere (die eine Hälfte der linken Nebenniere blieb übrig) und endlich eine Kontrolloperation ohne Nebennierenentfernung. Die operierten Tiere wurden innerhalb von 2–4 h nach der Operation bestrahlt. In einem anderen Versuch wurden beide Nebennieren im Abstand von 1,4 und 7 Tagen nach der Bestrahlung entfernt, parallel wurden Kontrolloperationen durchgeführt. Bestrahlungsbedingungen: 180 kV; 1 mm Al, 0,5 mm Cu; 60 r/min; Gesamtdosis 620 r, mit Mekapion gemessen. In einigen Versuchen wurde Desoxycorticosteronacetat in der Menge von 0,25 mg per Maus und Tag oder Cortisonacetat in der Menge von 0,25 mg und 0,5 mg per Maus und Tag verabreicht. Die Leber solcher Tiere, die zwischen dem 7.–10. Tag nach Bestrahlung eingingen, oder die am 10. Tag getötet worden sind, wurde auf ihren Fettgehalt geprüft. Die Gesamtfette wurden durch Alkohol-Äther-Mischung (3:1) wiederholt extrahiert, durch Chloroform reextrahiert und nach Verbrennung in bekannter Menge von Bichromat-Schwefelsäurelösung nach Kibrick[5] durch iodometrische Titration bestimmt. Der Fettgehalt der Leber wurde in Prozenten des frischen Lebergewichtes angegeben.

[5] A. C. Kibrick und S. J. Skupp, Arch. Biochem. Biophys. *44*, 134 (1953).

Die Resultate unserer Versuche sind in Tabellenform wiedergegeben.

Der erste Versuch (Tab. I) zeigt, dass die Adrenalektomie die Leber, wie schon früher festgestellt wurde, vor der Entstehung der Fettinfiltration schützt. Dagegen ist die eine Hälfte der Nebenniere völlig ausreichend, um den Verlauf dieser Reaktion zu ermöglichen.

Die Resultate des zweiten Versuches zeigen (Tab. II), dass auch die erst nach der Bestrahlung durchgeführte Adrenalektomie die Vermehrung von Leberfetten hemmt. Nur bei Mäusen, die am 7. Tage adrenalektomiert wurden, sieht man eine mässige Zunahme der Leberfette, welche wahrscheinlich schon vor dem 7. Tage stattgefunden hat.

Aus den weiteren Versuchen (Tab. III) geht hervor, dass die Verabreichung kleiner Mengen von Cortison imstande ist, die Fähigkeit adrenalektomierter Tier zur Vermehrung von Leberfetten zu erneuern. Die benutzten Mengen verursachen dagegen sowohl bei unbestrahlten Tieren wie auch bei bestrahlten Mäusen mit beiden Nebennieren keine Veränderungen im Fettgehalt der Leber.

Nach dem letzten Versuch können wir den histologischen Befund von Ellinger[1], wonach die Verabreichung von Desoxycorticosteron die Fettinfiltration der Leber nach der Bestrahlung vermindert, nicht bestätigen. Wir finden nämlich in unserem Versuch keinen Unterschied im Fettgehalt der Leber zwischen den beiden Versuchsgruppen (Tab. IV).

* In allen Tabellen sind die Mittelwerte mit Standardabweichungen und Anzahl der Tiere (in Klammern) angegeben.

Tabelle II
Leberfettgehalt bei bestrahlten Mäusen (Männchen), Adrenalektomie nach der Bestrahlung

	Durchführung der Operation		
	1. Tag	4. Tag	7. Tag
Unbestrahlte Kontrolltiere		3,9 ± 0,60 (8)	
Bestrahlte Kontrolltiere	7,7 ± 1,39 (5)	9,1 ± 1,86 (5)	8,6 ± 2,05 (5)
Bestrahlte adrenalektomierte Tiere	4,4 ± 1,82 (10)	4,2 ± 1,69 (13)	5,0 ± 1,50 (15)

Tabelle III
Leberfettgehalt bei bestrahlten Mäusen (I Männchen, II Weibchen), mit Cortison behandelt

		Behandlung mit Cortisonacetat		
		ohne	0,25 mg/Tag	0,5 mg/Tag
I	Unbestrahlte Kontrolltiere	4,1 ± 0,75 (7)	4,1 ± 0,29 (8)	4,3 ± 0,43 (8)
	Bestrahlte Kontrolltiere	8,3 ± 2,90 (7)	7,7 ± 2,29 (8)	8,1 ± 2,66 (7)
	Unbestrahlte adrenalektomierte Tiere	3,9 ± 0,26 (8)	4,7 ± 1,18 (8)	4,3 ± 0,69 (8)
	Bestrahlte adrenalektomierte Tiere	4,2 ± 1,27 (8)	5,8 ± 1,92 (8)	8,8 ± 2,49 (7)
II	Unbestrahlte Kontrolltiere	3,3 ± 0,70 (7)	3,2 ± 0,36 (7)	3,5 ± 0,83 (7)
	Bestrahlte Kontrolltiere	7,1 ± 1,90 (7)	8,0 ± 1,45 (7)	6,6 ± 1,77 (7)
	Unbestrahlte adrenalektomierte Tiere	3,0 ± 0,55 (8)	3,1 ± 0,35 (7)	3,1 ± 0,73 (9)
	Bestrahlte adrenalektomierte Tiere	3,4 ± 0,76 (8)	7,2 ± 1,16 (8)	8,3 ± 1,82 (8)

Die Versuchsresultate führen uns zu folgenden Schlussfolgerungen: Zur Entstehung der Fettinfiltration der Leber nach Bestrahlung ist das Vorhandensein von wenigstens einem kleinen Teil der Nebennierenrinde notwendig, was schon früher mit Rücksicht auf die Wirkung von verschiedenen Noxen[6] festgestellt wurde. Zugabe von Cortison kann die Nebennierenrindentätigkeit ersetzen, wie dies auch bei der Fettinfiltration der Leber durch Alkoholvergiftung der Fall ist[7].

Wir kommen deshalb zur Ansicht, dass die Nebennierenrinde mit ihren Hormonen im Fall der Fettinfiltration nach Bestrahlung nur die Rolle eines Vermittlers spielt, welche metabolische Wege zur Fettvermehrung in der Leber aus anderen Gründen ermöglicht. Nach unseren Versuchen ist es nicht möglich, die Verfettung der Leber als Folge der Störung der Nebennierenrindenfunktion durch Bestrahlung aufzufassen.

M. Skalka

Biophysikalisches Institut der Tschechoslowakischen Akademie der Wissenschaften, Brno, 14. Oktober 1958.

Summary

The presence of adrenals is necessary for the genesis of the fatty liver damage in the second week after the total-body X-irradiation. Cortison in moderate daily doses is capable of replacing this function of the adrenals in adrenalectomized mice.

[6] E. M. MacKay und H. O. Carne, Proc. Soc. exp. Biol. Med. *38*, 131 (1938). – F. Verzár und L. Laszt, Biochem. Z. *288*, 356 (1936).
[7] S. Mallow und J. L. Bloch, Amer. J. Physiol. *164*, 29 (1956).

Die Infektiosität des Ribonukleinsäureanteils von Maul- und Klauenseuche-Virus

Nachdem es im Jahre 1956 Gierer und Schramm[1,2] sowie 1957 Fraenkel-Conrat, Singer und Williams[3] gelungen war, das Virus der Tabakmosaikkrankheit in infektiöse Ribonukleinsäure (RNS) und einen apathogenen Proteinanteil zu trennen, ist in kurzer Aufeinanderfolge auch über die Gewinnung von infektiöser RNS aus verschiedenen tierpathogenen Viren berichtet worden[4,5].

So haben Brown, Sellers und Stewart Doreen[6] vor kurzem aus dem Maul- und Klauenseuche-Virus (MKS) RNS-Präparate erhalten können, die sich für die säugende Maus und für Kulturen von Schweinenieren-Zellen als infektiös erwiesen.

Ganz analoge Versuche sind von uns unternommen worden, nachdem es sich gezeigt hatte, dass für die Aufteilung in RNS-Anteil und Protein von verhältnismässig wenig gereinigten Viren ausgegangen werden kann[4,5].

Als Ausgangsmaterial für die Gewinnung infektiöser RNS aus MKS-Virus benützten wir Zungenaphthen von künstlich mit MKS-Virus infizierten Rindern. Zur Infektion der Rinder waren die Stämme Spanien II (0-Typ), Eystrup (A-Typ) und Tölz (C-Typ) verwendet worden. Es kamen sowohl frisch gewonnene Zungenaphthen als auch solche, die während 9 Monaten bei −40° aufbewahrt worden waren, zur Verwendung. Die Virus enthaltenden Zungenepithelien wurden mechanisch fein zerkleinert und hierauf mit 0,02 *M* Phosphat-Puffer (pH 7,5–7,6) im Verhältnis 1:4 aufgenommen, anschliessend 30 min bei 8000 T/min zentrifugiert. Die so gewonnene Virussuspension wurde mit dem gleichen Volumen Chloroform durch kräftiges mechanisches Rühren homogenisiert, anschliessend zentrifugiert, abdekantiert und die überstehende Lösung während 24 h bei +4° aufbewahrt.

Die Gewinnung infektiöser RNS aus dieser Virussuspension geschah sodann mit der Phenolmethode nach Gierer und Schramm[1,2].

Der RNS-Anteil aller 3 Virustypen (0, A und C) erwies sich sowohl auf Rind wie auf Meerschweinchen als infektiös. Das in diesen Tieren gebildete Virus liess sich in Passagen weiterzüchten und zeigte jedesmal typenspezifisch an (Komplementbindungsreaktion).

Der absolute Beweis des Fehlens von restlichen Viruspartikeln in unseren RNS-Präparaten konnte noch nicht erbracht werden. (In jedem Versuch wurden 3–5 Phenolextraktionen und anschliessend 4–5 Ätherextraktionen ausgeführt.)

Immerhin deuten folgende Prüfungen auf das Fehlen von komplettem Virus in unseren infektiösen RNS-Lösungen hin:

1. Nach Behandlung mit Ribonuklease in einer Konzentration von 10–20 μg pro ml verliert der RNS-Anteil vollständig seine Infektiosität (Kontaktzeit 15 min bei +4°). Eine Viruskontrollsuspension, die sogar auf 10^{-4} verdünnt worden war, behielt unter gleichen Bedingungen ihre Infektiosität vollständig bei.

2. Erwärmung während 30 min auf 37° vernichtet die Aktivität der RNS. Eine Viruskontrollsuspension (Verdünnung 10^{-4}) zeigte unter gleichen Bedingungen keine Einbusse der Infektiosität.

3. Die RNS-Lösungen verlieren ihre Wirksamkeit sehr rasch. Eine Lösung erwies sich nach 4 Tagen bei −4° nicht mehr als infektiös. Eine weitere RNS-Lösung zeigte schon nach 48 h bei +4° keine Wirksamkeit mehr. Nach 24 h bei +4° erwies sich ein RNS-Präparat noch als infektiös, jedoch in erheblich reduziertem Masse. Suspensionen von komplettem Virus der MKS bleiben auch in starken Verdünnungen bei obigen Temperaturen und in gleichen Zeitintervallen noch stark infektiös.

V. Spuhler

Eidg. Veterinäramt, Bern, und Eidg. Vakzine-Institut, Basel, 29. Dezember 1958.

Summary

Using the phenol method described by Gierer and Schramm, preparations of infective ribonucleic acid were prepared from tongue tissues of cattle infected with the virus of foot-and-mouth disease.

[1] A. Gierer und G. Schramm, Nature *177*, 702 (1956).
[2] A. Gierer und G. Schramm, Z. Naturforsch. *11b*, 138 (1956).
[3] H. Fraenkel-Conrat, B. Singer und R. C. Williams, Biochim. biophys. Acta *25*, 87 (1957).
[4] J. S. Colter, *Progr. med. Virol.*, vol. 1 (Karger, Basel/New York 1958), p. 1–35.
[5] E. Wecker und W. Schäfer, Z. Naturforsch. *12b*, 415 (1957).
[6] F. Brown, R. F. Sellers, and Doreen L. Stewart, Nature *182*, 535 (1958).

Induction of Lactation in Precancerous Hyperplastic Alveolar Nodules in the Mammary Gland of C3H/He Crgl Mice

The hyperplastic alveolar nodule in the mammary gland of the C3H/He Crgl mouse is a precancerous lesion that is morphologically indistinguishable from a normal

lobule of prelactating alveoli characteristic of the mammary gland of pregnancy[1]. However, the nodule is distinct from the normal lobule in its possession of large numbers of virus-like particles[2] and in its hormonal sensitivity[3,4]. The nodule in a pregnant mouse will lactate prematurely.

Two kinds of experiments have been conducted on adult multiparous tumor-bearing mice whose mammary glands contain numerous hyperplastic nodules, in order to examine the reactivity of the nodules to hormones. In the first series of experiments, intact mice were subjected to month-long treatment with (a) 3–4 mg pellet of estradiol-17β implanted subcutaneously, (b) 1 mg progesterone and 2·5 μg estradiol injected daily, (c) 0·25 mg cortisol acetate injected daily, (d) 0·25 mg desoxycorticosterone acetate injected daily, (e) 1 mg testosterone injected daily. Groups of 10–20 mice were employed, and the mammary glands were compared before fixation and after preparation as whole-mounts stained with hematoxylin, with those of untreated controls.

All treatments employed resulted in a degree of stimulation of at least some hyperplastic nodules. Nodules underwent significant hypertrophy as a result of administration of estrogen, estrogen with progesterone, and cortisol; individual alveoli became dilated with secretion. Lactogenesis was evident in many nodules after cortisol treatment (Fig. *B*), in parts of some nodules after estrogen (Fig. *C*), but not at all after administration of both ovarian steroids (which induced extensive lobuloalveolar development throughout the entire mammary apparatus) (Fig. *D*). All nodules were stimulated (as judged by alveolar size) by the ovarian steroids; however, semi-regressed nodules were found adjacent to considerably hypertrophied nodules after cortisol treatment. In two of the 10 androgen-treated mice, some nodules with milk were seen; however, stained preparations revealed only occasional evidence of nodule stimulation, and some nodules had obviously partly regressed. No milk secretion, moderate stimulation of some nodules, and partial regression of others occurred after desoxycorticosterone administration. Nodules in untreated control mice (Fig. *A*) may occasionally show an alveolus or two with milky-appearing secretion. It should be emphasized that detection of milk formation was possible only by examination of fresh material; it has not proven feasible to decide from the stained whole-mounts alone whether or not the intense secretory activity evident in some nodules is accompanied by milk formation.

Nandi[5] has shown that properly prepared mammary glands in hypophysectomized-ovariectomized-adrenalectomized female C3H/He mice can be made to lactate by administration of cortisol along with either ovine mammotropin or bovine somatotropin (purified pituitary hormones generously provided by Professor C. H. Li). If the nodule be considered as a lobule of prelactational alveoli in a non-pregnant mouse approximately equivalent to its normal counterpart in pregnancy[1,6], one might expect that lactogenic hormonal combinations would result in milk formation. In a second series of experiments, small groups (3 to 9) of multiparous tumor-bearing mice were hypophysectomized, ovariectomized and adrenalectomized. In the absence of replacement therapy, the nodules, as well as the normal gland, will regress, and alveoli are not detectable after 21–30 days[3]. A 'ghost' of the nodule can often be found, consisting of its atrophic terminal duct system.

When 125 μg cortisol acetate are given along with 1 mg of mammotropin *or* somatotropin daily immediately after the triple operation, most of the recognizable alveolar nodules are found to be lactating 21 days later (Fig. *E–G*). As is the case with normal lobules in the C3H/He Crgl mouse[5], somatotropin is as effective as mammotropin in inducing lactogenesis in alveolar nodules (Fig. *F*). If the protein hormone dosage is reduced to 100 μg daily, full lactation is seen in only a few nodules and in parts of other nodules. Many nodules are clearly milk-free. If 250 μg desoxycorticosterone acetate are used daily with 1 mg somatotropin (but not mammotropin), a few nodules are found to be partially lactating; the overall picture resembles that seen after cortisol and the 100 μg protein hormone treatment. Nodules are thus seen to show a spectrum of sensitivity to various lactogenic combinations, and in the case of desoxycorticosterone and somatotropin, to respond to a combination that does not result in lactogenesis in normal prelactating C3H/He mouse tissue[5].

It can be postulated that the lactogenic effect of certain steroids (estradiol and testosterone) in intact mice results from the release of a pituitary factor, presumably mammotropin, that then acts in the presence of adequate endogenous corticosteroid. It is also possible that estrogen may result in increased corticoid secretion by triggering ACTH release[7]. The lactogenic effect of cortisol may be ascribed to its direct action in the presence of endogenous mammotropin and/or somatotropin[4,5]. The failure to observe lactation after estrogen and progesterone in intact mice, despite extensive general lobuloalveolar development, may indicate an inhibition of mammotropin release by progesterone or some effect mediated by the adrenal cortex.

The hyperplastic alveolar nodule represents a special test area for lactogenic combinations. In intact mice, the nodules not only lactate normally during pregnancy and *post partum*, but can be induced to lactate most effectively by the lactational triad of mammotropin, somatotropin, and cortisol that Lyons and Li and their collaborators have found most effective in the Long-Evans rat[8]. It is proving possible to construct a kind of hormonal 'sieve' by using graded doses of lactogenic hormones and thus to arrive at a picture of differential nodule sensitivity. We are presently concerned with the determination of the relationship between the hormonal sensitivity of nodules and their predisposition to neoplasia, by employing a combination of graded hormonal stimuli and subsequent transplantation in accordance with a technique recently developed in our laboratory by DeOme and Faulkin.

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H. A. Bern, S. Nandi, and V. Finster

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[2] D. R. Pitelka, H. A. Bern, K. B. Deome, C. N. Schooley, and S. R. Wellings, J. Nat. Cancer Inst. *20*, 541 (1958).

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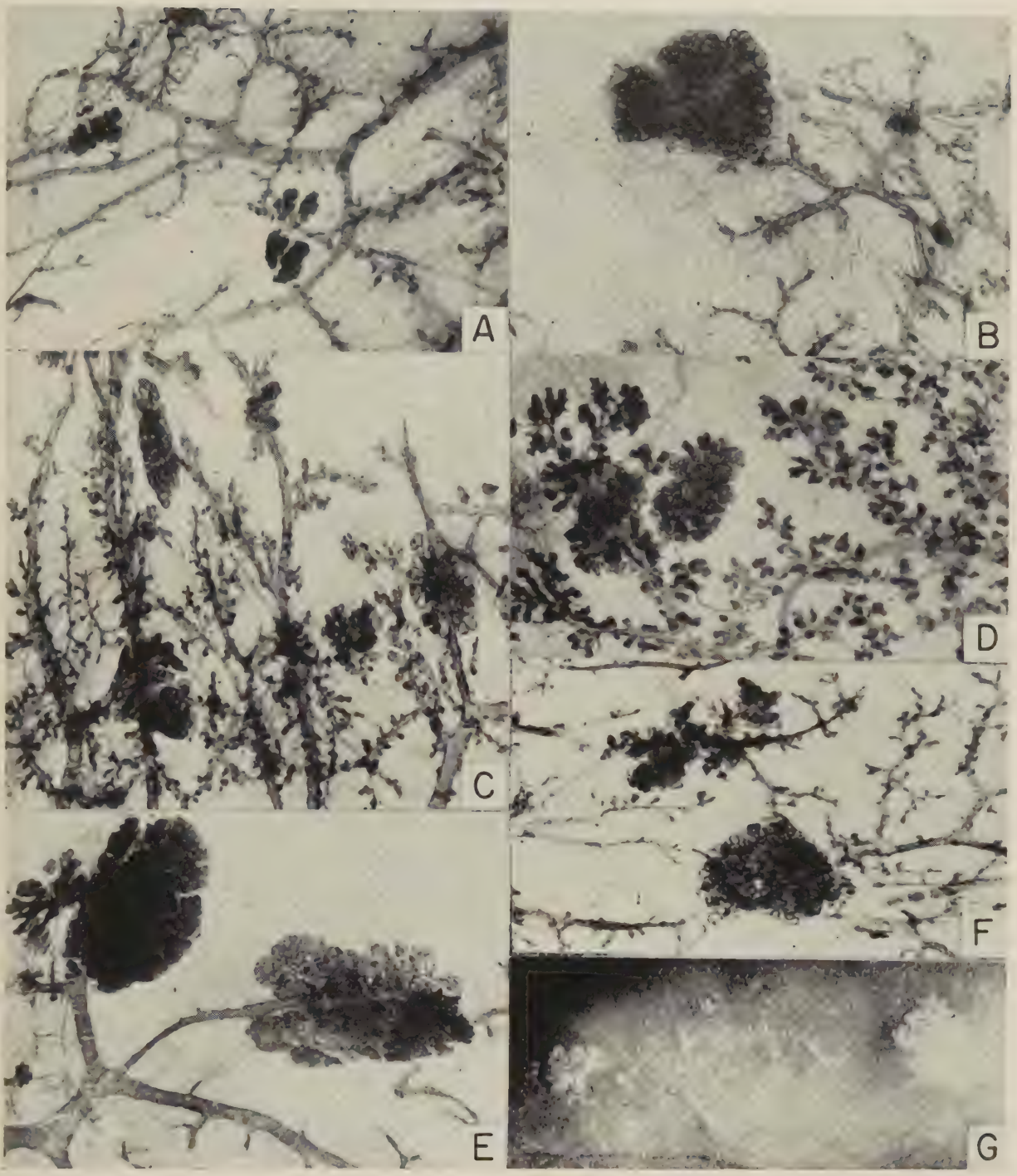


Hyperplastic alveolar nodules in mammary glands of variously treated multiparous female C3H/He Crgl mice. *A–F* are whole-mounts, stained with hematoxylin, ×15; *G* was photographed from freshly fixed material, ×10. *A*, small nodules from untreated non-pregnant mouse; *B*, from cortisol-treated intact mouse (lactating nodule); *C*, from estrogen-treated intact mouse (partially lactating nodules); *D*, from estrogen + progesterone-treated intact mouse (non-lactating nodules); *E* and *G*, from hypophysectomized-ovariectomized-adrenalectomized mouse treated with 1 mg mammotropin and 125 μg cortisol daily (lactating nodules); *F*, from hypophysectomized-ovariectomized-adrenalectomized mouse treated with 1 mg somatotropin and 125 μg cortisol daily (lactating nodules).

Department of Zoology and its Cancer Research Genetics Laboratory, University of California, Berkeley, September 17, 1958.

Résumé

La sécrétion lactée se produit dans les nodules alvéolaires hyperplastiques de la glande mammaire des souris femelles intactes de la souche C3H/He Crgl, à la suite de l'administration du cortisol et de l'œstradiol, mais non pas après l'emploi de l'œstrogène avec le progestérone. Lorsque l'hypophyse, l'ovaire et la glande surrénale ont été enlevés, les nodules montrent une sécrétion lactée sous l'effet du cortisol et de la mammotropine ou de la somatotropine et indiquent une sensibilité différentielle aux combinaisons hormonales lactogènes.

PRO EXPERIMENTIS

The Interference of Phenols in the Fluorometric Determination of Nucleotides

Lowry *et al.*[1] described a fluorometric method by which both oxidised and reduced phosphopyridine nucleotides, DPN, TPN, DPNH, and TPNH could be determined in animal tissues. An attempt was made to apply this method to plant tissue in view of the importance of a knowledge of such ratios in plants.

Homogenates of etiolated pea or lettuce seedlings were prepared in tris buffer and analysed by the method of Lowry. No nucleotides could be detected. In recovery experiments where DPN was added to the homogenates, a loss of DPN of between 30 and 60% was observed. This loss was apparently not due to enzymic destruction since it was observed also when extracts of pea tissue were prepared in ice cold methanol and even after boiling such extracts. It appeared likely that some compounds in the extracts were interfering either with the development of fluorescence of the nucleotides during alkali treatment or were acting as quenching agents. Phenolic substances are widely present in plant tissue and are known to form oxidation products in strong base which might be responsible. A number of such compounds were therefore tested. The compounds were added to the DPN solution either before adding alkali and keeping at 38° C for 1 h or immediately before dilution for reading the intensity of fluorescence. Some of the results obtained are given in the Table. The recovery of DPN is calculated from the expected and observed fluorescence. An Eel fluorimeter having a primary filter transmitting at 365 mμ and a secondary filter with a transmission peak at 465 mμ was used. All tests were made in a range where the fluorescence of DPN and quinine sulphate is linear with concentration.

Effect of various substances on the fluorescence of DPN at a final concentration 1 μg/ml

Compound	Concentration	% Recovery of DPN if compound added	
		Before adding alkali	Before dilution
Catechol	2×10^{-4} *M*	63	62
Chlorogenic Acid	$1{\cdot}1\times10^{-4}$ *M*	100	—
Phloroglucinol	2×10^{-4} *M*	76	77
Hydroquinone	2×10^{-4} *M*	65	61
Dopa	1×10^{-4} *M*	—	63
Caffeic acid	$1{\cdot}1\times10^{-4}$ *M*	—	90
Catechol + Hydroquinone each	2×10^{-4} *M*	49	—
Tyrosine	$1{\cdot}1\times10^{-4}$ *M*	100	—
Resorcinol	2×10^{-4} *M*	100	—
2,4-dihydroxy benzoic acid	$1{\cdot}3\times10^{-4}$ *M*	100	—

It will be seen from this Table that a number of compounds are capable of producing an apparent loss of DPN. This appears to be due to a quenching effect in the case of catechol and phloroglucinol and hydroquinone. Whether quenching occurs or not is clearly related to the structure of the phenolic compound tested. It is particularly worth nothing that even compounds which fluoresce themselves such as DOPA and caffeic acid can cause an apparent reduction of the fluorescence of DPN in strong alkali. The fluorescence is clearly not additive.

It must be concluded that the method of Lowry *et al.*[1] cannot be applied to any tissue where such phenolic compounds are likely to occur, especially since the amounts of and ratios between various phenols in plant tissues are liable to change markedly during development of the plant.

I would like to thank the Superintendent of the Low Temperature Research Station for providing the facilities for carrying out this work.

A. M. Mayer

Low Temperature Research Station, Cambridge, England, Dezember 8, 1958.*

Zusammenfassung

Die fluorometrische Bestimmung von Nukleotiden in Pflanzenextrakten wird durch phenolische Stoffe gestört. Nukleotide können mit dieser Methode nicht routinemässig bestimmt werden.

* Permanent Address: Botany Department, Hebrew University, Jerusalem (Israel).

[1] O. H. Lowry, N. R. Roberts, and J. I. Kapphann, J. biol. Chem. *224*, 1047 (1957).

PRO EXPERIMENTIS

Vergleichende röntgenhistoradiographische und interferenzmikroskopische Trockengewichtsbestimmungen[1]

Von Engström und Lindström[2] wurde die Röntgenhistoradiographie mit weichen Röntgenstrahlen zur Trokkengewichtsbestimmung an kleinsten biologischen Objekten eingeführt. Sie stellt neben der Interferenzmikroskopie eine wichtige unabhängige Methode für diese Zwecke dar. Davies *et al.*[3] untersuchten erstmals vergleichend mit beiden Verfahren verschiedene Objekte und fanden eine gute Übereinstimmung der Messergebnisse.

Wir benutzten in unseren röntgenhistoradiographischen Untersuchungen das Gerät von Philips (CMR 5). Wegen des kleinen ausgeleuchteten Feldes können hier Objekt und Referenzsystem kaum gleichzeitig photographiert werden. Wir mussten deshalb eine Methode entwickeln, die es gestattet, Trockengewichtsbestimmungen ohne Referenzsystem durchzuführen. Dazu wurde die Zeit-Schwärzungskurve für den verwendeten Film (linear bis $D = 0{,}7$ für Kodak Maximum Resolution Plates bei Entwicklung mit Gevaert G 209 A, 6 min, 18°C) bei konstanter Spannung festgelegt und mit einem Referenzsystem ein Trockengewichtsäquivalent bestimmt. (Für Einzelheiten der Methodik siehe Müller *et al.*[4], im Druck.)

Die Objekte (Erythrozyten von *Rana temporaria*, Bullenspermien, Bullenthymuslymphozyten, Plattenepithelien der menschlichen Mundschleimhaut) wurden auf Prä-

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.

[2] A. Engström und B. Lindström, Nature *163*, 563 (1949); Biochim. biophys. Acta *4*, 351 (1950).

[3] H. G. Davies, A. Engström und B. Lindström, Nature *172*, 1041 (1953).

[4] D. Müller, W. Sandritter und G. Schwaiger, Histochemie (im Druck).

Vergleichende röntgenhistoradiographische interferenzmikroskopische Trockengewichtsbestimmungen

Zellart	Röntgenhistoradiographische Messungen		Interferenzmikroskopische Messungen	
	Zellzahl	Trockengewicht in 10^{-12} g	Zellzahl	Trockengewicht in 10^{-12} g
Bullenspermien	16	7,87 ± 0,46	162	8,94 ± 1,17 [5]
Bullenthymuslymphozyten	26	15,05 ± 0,53	96	14,40 ± 1,58 [5]
Froscherythrozyten				
Kern	49	24,00 ± 0,69	26	21,55 ± 0,84 [5]
Kern	18	21,29 ± 0,78	6	20,00 ± 0,72 [5]
Plasma	29	89,24 ± 5,40	–	– –
Plattenepithel der Mundschleimhaut				
Kern	5	77,80 ± 4,62	8	58,00 [8]
Kern	22	70,36 ± 4,26	38	55,50 ± 2,6 [6]
Plasma	27	1068,00 ± 84,00	8	2272,00 ± – [8]
Plasma	—	– –	37	1941,20 ± 61,5 [6]

[5] W. Sandritter, D. Müller und H. G. Schiemer, Anat. Anz., 1959 (im Druck).

[6] W. Sandritter, H. G. Schiemer, W. Alt, D. Müller und E. Behrouzi, Frankf. Z. Path. *69*, 167 (1958).

parateträgern, die mit Mylarfolien bespannt waren, ausgestrichen. In allen Fällen, ausser den Mundschleimhautepithelien, kamen die gleichen Objekte mit beiden Methoden zur Untersuchung. Bei einer Spannung von 1,52 kV, 1,5 mA Röhrenstrom und 30 min Belichtungszeit wurden mit dem Historadiographen CMR 5 auf Kodak Max. Res. Plates Röntgen-Kontaktaufnahmen angefertigt und 6 min in G 209 A Gevaert bei 18°C entwickelt. Die Auswertung der Kontaktaufnahmen erfolgte mit einem Zytophotometer (Sandritter *et al.*[7]) mit einer Messblende von 5 μ^2. Die interferenzmikroskopischen Untersuchungen wurden mit der Analysatormethode durchgeführt (Bakersches Interferenzmikroskop, vgl. Schiemer *et al.*[8]).

Das Ergebnis der röntgenhistoradiographisch und interferenzmikroskopisch gewonnenen Trockengewichte zeigt die Tabelle. Die Messwerte zeigen eine gute Übereinstimmung. Die Abweichungen sind durch den systematischen Fehler beider Methoden bedingt. Diese Ergebnisse scheinen uns deshalb bemerkenswert, weil nicht nur die Genauigkeit der interferenzmikroskopischen und röntgenhistoradiographischen Technik aufgezeigt, sondern damit auch die von uns eingeführte Methodik der röntgenhistoradiographischen Trockengewichtsbestimmungen ohne routinemässige Verwendung eines Referenzsystems gesichert wird. So ist es erstmals möglich geworden mit dem einfachen Gerät CMR 5 quantitative Röntgenhistoradiographie bei geringem Arbeitsaufwand zu betreiben.

W. Sandritter und D. Müller

Senckenbergisches Pathologisches Institut der Universität Frankfurt a. M., 10. November 1958.

Summary

Quantitative roentgen-histo-radiographic dry-weight determinations were made without a reference system. The results were compared to readings which had been obtained by the interference microscope. The readings from both methods correspond. The results show the range of error for both methods. It is remarkable that roentgen-histo-radiographic dry-weight determinations were made without using a reference system, once the dry-weight equivalent had been established.

[7] W. Sandritter, W. Mondorf, H. G. Schiemer und D. Müller, Mikroskopie (im Druck).

[8] H. G. Schiemer, W. Alt und W. Sandritter, Acta Histochem. *4*, 325 (1957).

PRO EXPERIMENTIS

A Method of Reducing the Fibrinolytic Activity of Endometrium Cells in Plasma Clot Tissue Culture

The difficulty of preventing growing cells from digesting plasma coagel has been a problem since the earliest days of tissue culture. Repeated efforts have been made to overcome this obstacle (Losee and Ebeling[1], Carrel[2]). More recently, the trypsin inhibitor, isolated by Kunitz[3] from soya bean extract, has been used; but it was found to have a growth retarding effect and to prolong the coagulation time of the plasma (Fischer[4]).

Certain cells, especially those of the uro-genital system, contain a large quantity of fibrinolysins; consequently the cells liquefy the plasma clot so much that there has been great difficulty in growing this kind of cell in tissue culture, even for short-term studies. In studying the endocrine reactions of rat endometrium *in vitro*, the present author met with this problem of preventing fibrinolysis. However, the addition of lysine-ethyl-ester (LEe) to the clot has made it possible to prevent its digestion by the endometrial cells. Experiments carried out by Troll, Sherry, and Wachsman[5] have shown that esters of lysine and arginine are specific substrates for plasmin, the proteolytic enzyme of mammalian plasma. Aware of this fact, the present author used the LEe in the plasma clot of her experiments to prevent the fibrinolytic action of the epithelial cells of rat endometrium.

Method. Small slices of uterus from albino rats, spayed 4–6 weeks before the experiments, were explanted in Carrel flasks (D 5) in a solid phase containing 0·5 ml of cockerel plasma and 0·5 ml of 5% chicken embryonic extract in Tyrode's solution. In some cultures LEe was added to the extract, producing a final concentration in the clot of 1·25–12·5 mg/ml. This solution, however, was found to be acid (10 mg/ml of LEe in Tyrode has a pH value of 6·4). Therefore, as a control, a Tyrode solution

[1] J. R. Losee and A. H. Ebeling, J. exp. Med. *19*, 593 (1914).

[2] A. Carrel, J. exp. Med. *38*, 407 (1923).

[3] M. Kunitz, Science *101*, 668 (1945).

[4] A. Fischer, Science *109*, 611 (1949).

[5] W. Troll, S. Sherry, and J. Wachsman, J. biol. Chem. *208*, 85 (1954).

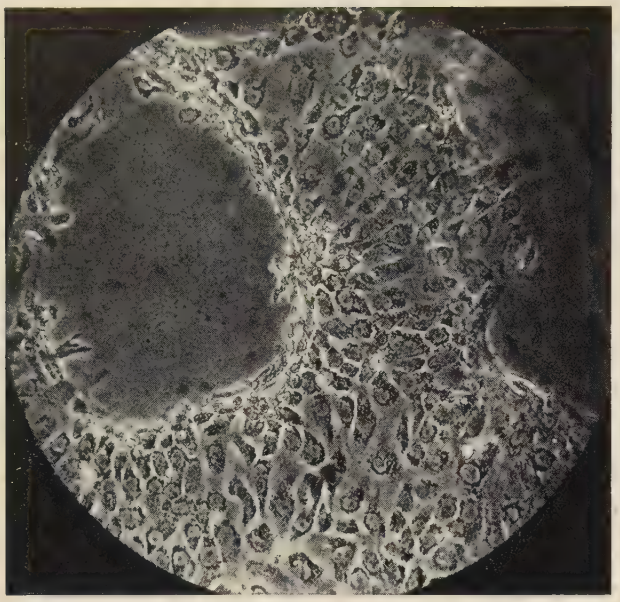


Fig. 1.—Endometrial cells, 5 days old, in control medium. Big vacuoles as a result of fibrinolysis; cells are somewhat dispersed. Magnification 110×

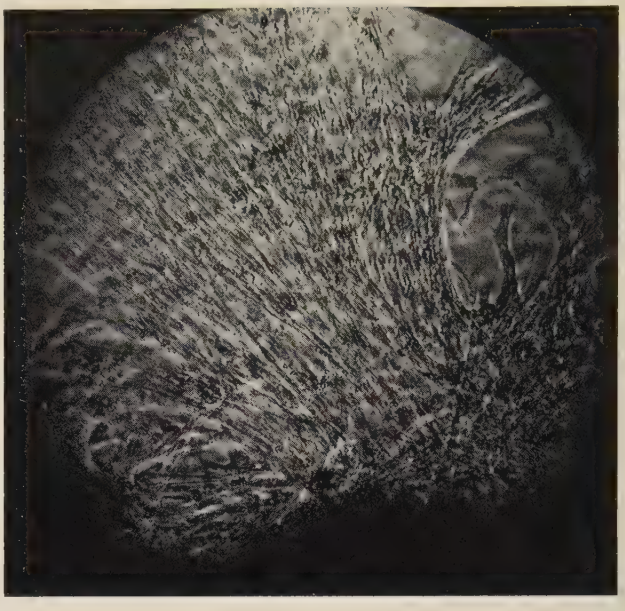


Fig. 3.—Fibroblastic outgrowth in control culture, 6 days old. Massive growth is apparent. Magnification 110×

was used with a pH adjusted to this level by reduction of bicarbonate. A third group consisted of cultures made with ordinary Tyrode's solution without LEe.

Results. After 2–3 days, the controls with ordinary Tyrode's solution showed an abundant outgrowth of epithelium containing big vacuoles as a consequence of fibrinolysis. On the 3rd and 4th day, many of the pieces became detached from the plasma clot due to the digestion by the cells. From the cultures still left in the clot, on the 4th and 5th days big vacuoles could be seen in the outgrowth as a result of the fibrinolysis (Fig. 1). Moreover, a massive invasive growth of fibroblasts into the epithelial cell sheets was often visible. The behaviour of the cells in the 'acid Tyrode' was identical with that of the controls, and the same picture could also be seen in the cultures with the lowest concentration of LEe (final conc. 1·25 mg/ml). In the tissues cultured in the presence of LEe solution in a final concentration of 2·5–5 mg/ml, a retarded outgrowth could be observed beginning after 3–4 days. It was not as abundant as in the control flasks but could be characterized as good. It formed large epithelial sheets without fibrinolysis, only a few vacuoles were seen, and no pieces became detached from the plasma clot (Fig. 2). The fibroblasts were remarkably arrested in their growth, and no invasion of these cells into the epithelial growth could be observed (Figs. 3–4). Few fibroblasts could be seen in these cultures, and they appeared to be toxically affected. The outgrowth of epithelium

Fig. 2.—Endometrial cells, 6 days old, in a medium containing LEe 2·5 mg/ml. Continuous sheet of closely packed cells. Magnification 110×

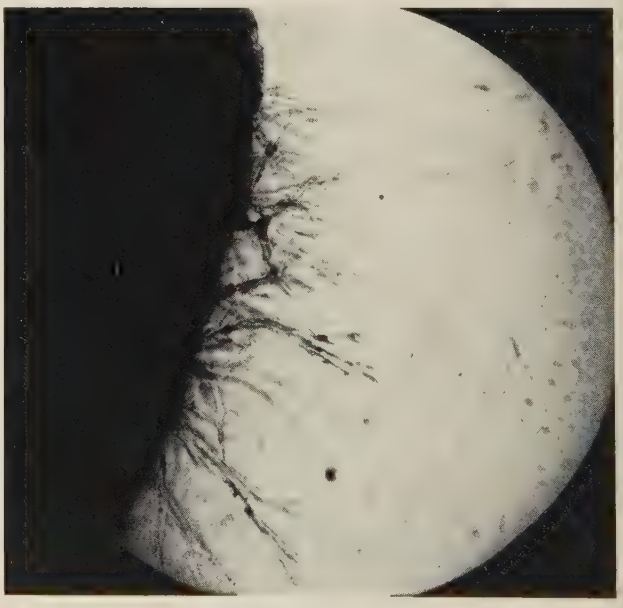


Fig. 4.—Fibroblastic outgrowth in a culture with LEe 2·5 mg/ml, 6 days old. Poor growth. Magnification 110×

went on to the 8th–9th day, after which it showed degenerative signs. In tissues cultured in a medium containing higher concentration of LEe (12·5 mg/ml), the outgrowth of cells was slight or totally inhibited.

In different series of this type, the various endometria show diverse degrees of fibrinolytic activity. In the controls, good epithelial outgrowth with only slight fibrinolysis can sometimes be observed. In other cases, a final concentration of LEe of 2·5 mg/ml is not enough to prevent fibrinolysis and digestion of the clot, but a concentration of 5 mg/ml or higher has a good effect.

Discussion. The effect of LEe in preventing the liquefaction of high fibrinolytic tissue in a plasma clot is probably due to its power to work as an inhibitor competitive with the protein substrates of plasmin. The power of LEe to prevent selectively the outgrowth of fibroblasts is also of great value in endocrinological studies of the epithelial cells. According to experiments in our laboratory, the LEe seems to be of great value in preventing the fibrinolysis in cultures with other tissues from the uro-genital system, which earlier proved very difficult or impossible to culture because of the intense fibrinolytic activity of the cells. Successful studies have thus been carried out with prostatic hyperplasia, human placenta, and different malignant tumours of the uro-genital system.

B. Ingemanson

Department of Embryology, University of Lund (Sweden), December 1, 1958.

Zusammenfassung

Durch Zusatz von Lysin-äthylester (LEe) zum Plasmakoagel in einer Konzentration von 2,5–5 mg/ml ist es möglich, die fibrinolytische Aktivität des Endometriums in Gewebezuchten zu verhindern. Ausserdem hemmt LEe das Fibroblastenwachstum.

PRO EXPERIMENTIS

Organtypische und phasenspezifische Verteilung ungesättigter Lipoide im Schwanz der Larve von *Xenopus laevis*

Von den Lipoiden in der Zelle ist bis jetzt nur bekannt, dass sie Bausteine wichtiger Zellorganoide, wie zum Beispiel der Mitochondrien, des hyaloplasmatischen Retikulums und der Kernmembran sind. Insbesondere die Phosphatide enthalten auch ungesättigte Fettsäuren. Alle fettartigen Substanzen sind relativ unspezifisch durch Sudan III oder Sudanschwarz nachweisbar. Eine histochemische Auftrennung der gesättigten und ungesättigten Komponenten ist bis jetzt nicht bekannt. Wigglesworth[1] vermutet, dass nur ungesättigte Komponenten durch Osmiumtetroxyd geschwärzt werden. Ihre Menge kann offenbar während verschiedener physiologischer Funktionszustände relativ stark und rasch wechseln, was auch die Versuche von George und Scaria[2] über die Verteilung von Lipasen im Muskel verschiedener Wirbeltiere bestätigen.

Herr Prof. Dr. Lehmann regte deshalb an, die Veränderungen im Gehalt ungesättigter Lipoide (u. L.) weiter zu verfolgen. Ein von Barrollier[3] für Papierchromatogramme beschriebener spezifischer Nachweis von ungesättigten Fettsäuren beruht darauf, dass an ungesättigten aliphatischen Bindungen mit Jodbromid Halogen angelagert und dieses mit Silbernitrat in Halogensilber überführt wird. Das Halogensilber wird mit einem Fotoentwickler (in unseren Experimenten stets Agfa 100) zu metallischem Silber reduziert. Diese Methode wurde von uns auf biologische Objekte übertragen und als histochemisches Reagens für ungesättigte Fettsäuren angenommen.

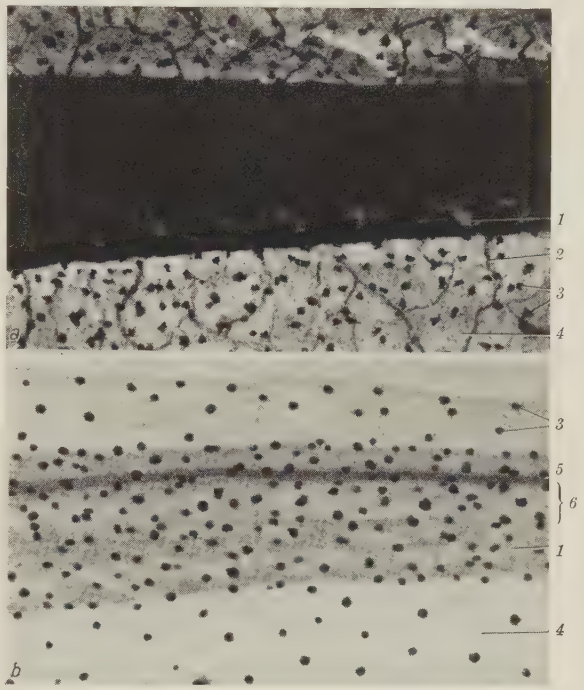


Abb. 1. *a*) IB2a. 40 Tage alte Larve. Starke Schwärzung von Muskulatur, Blutkörperchen und Mesenchymzellen. *b*) AgR41. 47 Tage alte Larve, die 17 Tage gehungert hat. Muskulatur und Blutkörperchen nicht geschwärzt, das Rückenmark dagegen noch sehr deutlich versilbert. 1. Myotome. 2. Blutgefässe mit versilberten Blutzellen. 3. Chromatophoren. 4. Schwarzgraue Mesenchymzellen. 5. Rückenmark. 6. Chorda. – Mikrofotos von Totalpräparaten, etwa 80fach.

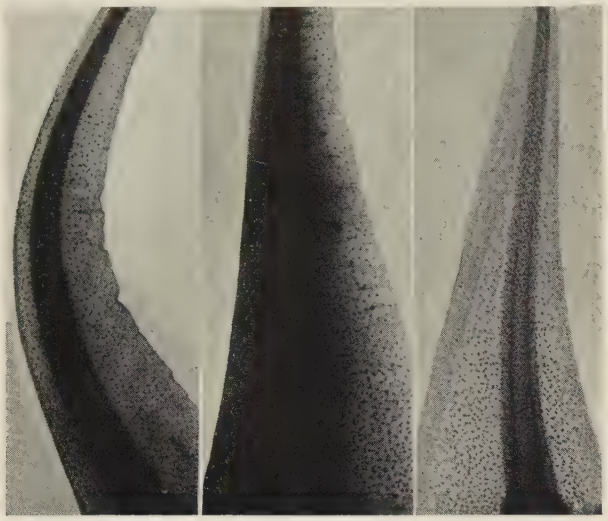
In Total- und Schnittpräparaten haben wir die differentielle Verteilung von ungesättigten Fettsäuren in den Organen des Schwanzes der *Xenopus*-Larve geprüft. Zunächst erkennt man eine *organtypische Verteilung* der u. L.: Die Muskulatur reagiert sehr stark. Das Zytoplasma der Blutzellen ist gleichmässig schwarzgrau, ihre Kerne sind völlig schwarz. Auch die Wände der Chordazellen sind deutlich geschwärzt. Nur wenig heller sind die verzweigten Mesenchymzellen in den Flossensäumen. Ihre Kerne heben sich weniger deutlich ab. Das Rückenmark wird gleichmässig dunkel gefärbt. Dagegen sind die Epidermiszellen mit Ausnahme einiger sehr feiner Granula frei von Silberablagerungen (Abb. 1 *a*). Eine gewisse *individuelle Schwankung* der Reaktion trat unabhängig von der Änderung der Schichtdicke der Gewebe auf und erschwerte so quantitative Aussagen.

[1] V. B. Wigglesworth, Proc. R. Soc. [B] *147*, 185 (1957).

[2] J. C. George and K. S. Scaria, J. Animal Morph. Physiol. *4*, 107 (1957); Nature *181*, 783 (1958); Naturwiss. *45*, 93 (1958).

[3] J. Barrollier, Naturwiss. *44*, 428 (1957).

Die phasenspezifische Änderung des Gehaltes an u. L. war deutlich. Die Schwänze von jungen Larven (bis zum Alter von etwa 20 Tagen bzw. bis ungefähr 20 mm Körperlänge) bleiben heller und enthalten wenig u. L. (Abb. 2*a*).



a *b* *c*

Abb. 2. Alterspezifische Reaktion auf ungesättigte Fettsäuren. *a*) AgN 4. 19 Tage alte Larve mit schwacher Reaktion. *b*) AgN 11. 43 Tage alte Larve mit sehr starker Reaktion. *c*) AgN 13. 50 Tage alte Larve, Beginn der Metamorphose, Schwärzungsreaktion wieder etwas schwächer. – Makrofotos von Totalpräparaten, etwa 8fach.

Am stärksten ist die Silberreaktion in Schwänzen von 25 bis 45 Tage alten Larven (30 bis 50 mm Körperlänge) (Abb. 2*b*). Schwänze noch älterer Larven (also schon während der Metamorphose) sind nach der Versilberung meist wieder heller (Abb. 2*c*). *Rasch- und langsamwachsende Larven* unterscheiden sich deutlich in ihrem Gehalt an u. L. Die Wachstumsgeschwindigkeit der *Xenopus*-Larven kann bei verschiedenen Eiablagen stark variieren. Schwänze gutwachsender Tiere geben eine kräftigere Reaktion, enthalten also mehr u. L. als die von langsamwachsenden.

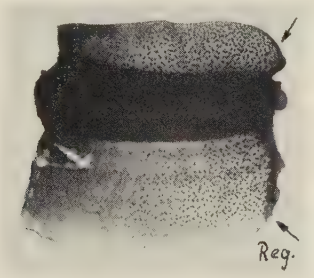


Abb. 3. IIA2a. 43 Tage alte Larve mit 3tägigem Regenerat, das deutlich stärker geschwärzt ist als der Stumpf. – Makrophotographie von Totalpräparat, etwa 12fach.

Der *Einfluss des Ernährungszustandes* auf den Gehalt an u. L. ergab sich aus Hungerversuchen. Während einer Hungerperiode nimmt der Gehalt an u. L. in den Larvenschwänzen stark ab (Abb. 1*b*). Am raschesten verliert dabei die Muskulatur ihre u. L. Sie gibt nach einer längeren Hungerperiode keine Schwärzungsreaktion mehr. Auch das Zytoplasma der Blutzellen wird im Laufe einer Hungerperiode immer heller, während ihre Kerne meist dunkler bleiben. Ebenso haben nach 20 oder mehr Hungertagen die Mesenchymzellen und die Wände der Chordazellen ihre u. L. verloren. Dagegen enthält das Rückenmark noch bis zum Hungertod (in unseren Experimenten zwischen dem 26. und 29. Hungertag) gut erkennbar u. L.

Am auffälligsten ist in allen Versuchen die starke Änderung des Gehaltes an u. L. in der Muskulatur. Diese Beobachtung ist analog zu den Befunden von George und Scaria[2], die in der Muskulatur verschiedener Wirbeltiere starke Schwankungen der Lipaseaktivität in Abhängigkeit von verschiedenen physiologischen Zuständen gefunden haben.

Das Enzymmuster im *regenerierenden Schwanzgewebe* ist anders als in normalem Gewebe[4], weshalb auch die Veränderung im Gehalt an u. L. nach Amputation der Schwanzspitze untersucht wurde. Unterschiede werden bereits 8 h nach der Operation erkennbar: Die miteinander verschmolzenen Epidermisränder an der Schnittfläche sind dunkler als das übrige Epithel (Abb. 3). Bis etwa zum achten Regenerationstag sind die Mesenchymzellen der Regenerate dunkler als im Stumpf.

Aus unseren Beobachtungen kann man allgemein schliessen, dass raschwachsende Gewebe reich an u. L. sind (gut gefütterte und raschwachsende Larven, Regenerate). Dagegen sind die Gewebe langsamwachsender oder hungernder Larven sowie beim Einschmelzen während der Metamorphose besonders arm an u. L. Zur Erklärung des oft sehr rasch erfolgenden Wechsels im Gehalt an ungesättigten Fettsäuren nehmen wir an, dass eine reversible Umwandlung von gesättigten in ungesättigte Fettsäuren möglich sei.

Ausgeführt mit Unterstützung der Eidgenössischen Kommission zur Förderung der wissenschaftlichen Forschung aus Arbeitsbeschaffungsmitteln des Bundes. Ich danke Herrn Prof. Dr. F. E. Lehmann für die Anregung zu dieser Arbeit und seine Unterstützung sowie Fräulein M. Pieren für technische Assistenz.

O. Hess

Zoologisches Institut und Theodor-Kocher-Institut der Universität Bern, 30. Dezember 1958.

Summary

Barrollier's method for unsaturated fatty acids (u.f.a.) in paper chromatograms has been adapted for cytochemical detection of u.f.a. in the tails of *Xenopus*-larvae. A specific pattern in the different organs was found. It was further shown that the content in u.f.a. changes quickly, apparently indicating rapid changes in physiological conditions during growth and regeneration.

[4] H. P. v. Hahn und F. E. Lehmann, Helv. Physiol. Acta *16*, 107 (1958).

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Relationship of Lateral Geniculate Activity to the Electrocorticogram in the Presence or Absence of the Optic Tract Input[1]

By G. B. Arden and U. Söderberg[2]

A series of similar light-stimuli to the eye produce variable responses in the lateral geniculate body[3], and the same is true of the medial geniculate[4]. It has been claimed, from neuroanatomical evidence, that almost all the nerve fibres afferent to the lateral geniculate originate in the retina[5]. Therefore a study of the activity of the lateral geniculate body, in relationship to the activity of other parts of the brain, should yield information about the effects of the retinal centrifugal system described by several authors[6].

We have tried to test this hypothesis by recording the activity of the lateral geniculate body before, during and after increasing the intra-ocular pressure (IOP) to the point where the eye became ischemic: this causes a reversible block of all retinal activity[7].

Experimental arrangements. Rabbits, anesthetized with ether, have been spinalized at C_1 and allowed to recover (encéphale isolé). One eye was eviscerated and the optic nerve head locally anesthetized with Xylocaine (Lidocaine). The other eye was stimulated with a flickering white light (Col. temp. 2760° K, intensity approximately 100000 lux). No precautions were taken to dark-adapt the animal.

The electrocorticogram (EEG) was recorded from Ag-AgCl electrodes placed on the dura. Lateral geniculate spikes were recorded by a glass micropipette filled with 4 *M* NaCl, tip resistance above 5 *MΩ*. Techniques for such recording and for identification of lateral geniculate cells are described by Arden and Liu[3]. Retinal activity was abolished by raising the IOP. This was achieved by connecting the eye to a constant pressure bottle via a small needle inserted into the anterior chamber. A second larger needle was inserted for IOP measurement.

We recorded on an inkwriter (Offner) and a cathode ray oscilloscope running synchronously. The spike discharges were integrated by an EKCO rate meter. Intraocular pressure (IOP) and arterial blood pressure (BP) were measured with strain gauge manometers (ELEMA).

The resting discharge and eye intact. Many cells discharged in bursts. The changes in average frequency in some cells were well correlated with changes in the EEG, the activity being higher when the animal was 'aroused'. Whistling and other noises also increased the activity, but there was no direct response to sound, the magnitude of whose effect was correlated with the effect produced in the EEG (Fig. 1 *A*). Sounds had little effect on the firing rate of the cell if the EEG already contained much high frequency activity. The animals were very sensitive to noise and the correlation described above only became clear if the loudspeaker which monitored the spikes was disconnected.

[1] One of us (G.B.A.) held an Alexander Piggot Wernher Memorial Fellowship in Ophthalmology. The research reported in this paper has in part been sponsored by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under Contract AF 61 (514)-1083.

[2] Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm (Sweden), January 13, 1959.

[3] R. L. de Valois, C. J. Smith, S. T. Kitai, and A. T. Karoly, Science *127*, 238 (1958). – G. B. Arden and Y.-M. Liu, in preparation.

[4] R. Galambos and V. G. Vernier, Amer. J. Physiol. *188*, 233 (1957).

[5] P. Glees and W. E. Le Gros Clark, J. Anat. *75*, 295 (1941). – S. Polyak, *The Vertebrate Visual System* (University of Chicago Press, Chicago 1957).

[6] S. Ramon y Cajal, *Histologie du système nerveux*, II, Chapter 15 (Madrid 1955). – R. Granit, J. Neurophysiol. *18*, 388 (1955). – E. Dodt, J. Neurophysiol. *19*, 308 (1956). – R. Hernández-Péon, H. Scherrer, and M. Velasco, Acta neurol. latinoamer. *2*, 8 (1956).

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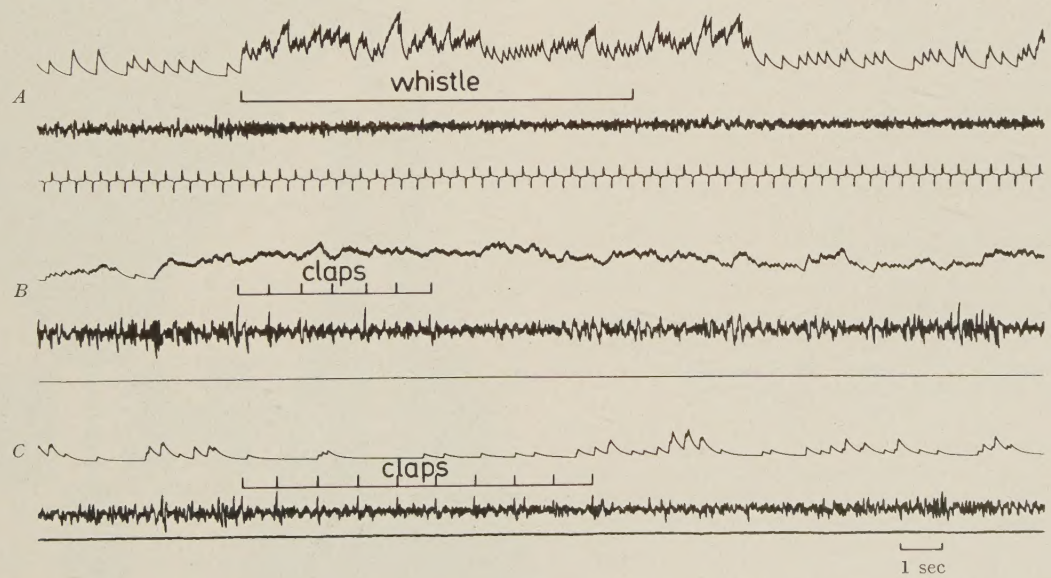

Fig. 1.—The relationship between rate meter record of geniculate unit activity, upper row; and EEG, middle record. Light and sound signals marked. *A*. IOP normal, light on. Soft whistle causes EEG activation and increases the discharge rate. *B*. IOP normal, no light. Handclaps arouse EEG and increase spike rate. *Note* evoked potentials in EEG at each clap. *C*. IOP raised, no light, no light reaction. Handclaps arouse EEG, but cause decrease in discharge rate.

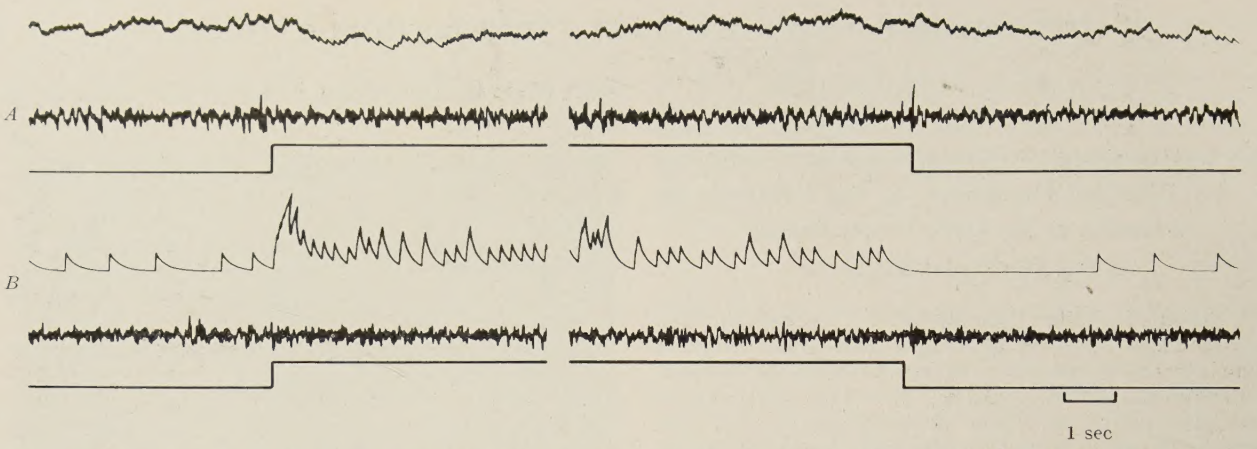

Fig. 2. – Records as in Fig. 1, from same rabbit. Both *A* and *B* show beginning and end of response to a flickering light-stimulus (as in Fig. 1 *A*). *A*. IOP normal – slight decrease in firing-rate at light on. *B*. Recovery following period of ocular ischemia – light now causes great increase in firing.

Light and the intact eye. The cells responded to flickering light by burst discharges similar to those seen in the resting discharge, but now phased with the flickering light. At the slow rate of flicker employed, there was often little difference between the overall rate of discharge in flickering light and in darkness (Fig. 2 *A*). At the beginning, and more commonly at the end of the flicker, transient changes in the average rate of discharge could occur. These were on the whole smaller than the 'spontaneous' changes in average rate. The average rate of discharge during a flicker was correlated with changes in the EEG, and with noise, in just the same way as was the resting discharge (Fig. 1 *B*). Sometimes noise increased the firing rate during a flicker, but not when the animal was in the dark.

In the animals studies, light had little effect in 'arousing' the EEG. Therefore, we cannot assess whether a light-stimulus that produces 'arousal' in the EEG, has the same effect on the response to light in the lateral geniculate body as one that does not.

Resting discharge, optic nerve blocked. When the IOP was increased above BP, after 3–5 min the response to light in the geniculate cell vanished. Before this occurred complex changes in the spontaneous discharge and evoked responses could occur. However, when the optic nerve was completely blocked the resting discharge remained, though its rate might be slightly decreased. In those cells whose activity was previously correlated to the EEG, correlation was still found, but was sometimes entirely reversed, so that now periods of fast activity in the EEG correlated with periods of low firing rate in the lateral geniculate cell, and periods of slow wave activity were associated with high firing rates. Similarly, noises such as whistling or clapping might cause an abrupt decrease in firing rate, associated with the appearance of fast activity in the EEG (Fig. 1 *C*). There were cells in which the correlation with the EEG only became apparent after blocking the input from the eye.

Recovery. When the IOP was returned to normal, the response to light rapidly returned. However, there was now a large increase in the average rate of firing during a period of flicker (Fig. 2 *B*); when full recovery had occurred, the initial conditions returned, and the average rate of firing was again, in some cells, almost the same in darkness and during flicker.

During the recovery, the relationship between EEG and spike discharge rate could reverse from that obtained during ischemia of the eye, to the original picture described above.

Nociceptive stimuli such as pinching the nose, were effective in 'arousing' the EEG. They usually caused a profound decrease in the firing rate. However, since the spike size was never constant, after nociceptive stimulation, it is not possible to exclude artefacts – movements or volume changes in the brain due to vasomotor changes[8].

Discussion. There are great variations in the behaviour of the different lateral geniculate cells we have studied. We wish to emphasize the *changes* that occur in response to arousing stimuli when the optic nerve is blocked. Since the average firing rate of the geniculate resting discharge is but little affected by the complete removal of the retinal afferents, and can be more affected by alterations in other parts of the brain than by alterations in retinal activity, there must be another afferent pathway to the lateral geniculate, at least as important as the optic tract. The alteration in relationship between EEG and geniculate discharge which we observe on raising the IOP may be due to the removal of a loop involving centrifugal retinal fibres. The alternative possibility remains of interneuronal interactions between the optic tract and the 'second input' at a level prior to the cell from which we record. There is some evidence that the lateral geniculate contains interneuronal paths[9]. The 'second input' may be identical with the fibres of the 'lame blanche intermédiaire'[10].

Zusammenfassung

Am Kaninchen wurde die Beziehung zwischen Elektrokortikogramm und Impulsentladungen der Einzelzellen des lateralen Knickes untersucht und die Befunde bei normaler Netzhauttätigkeit mit denen am entkoppelten Auge verglichen. Die reversible Entkoppelung der Netzhaut wurde mit intraokularer Drucksteigerung hervorgerufen. Die Spitzenpotentiale, die schon im Dunkeln gruppiert auftraten, waren im Flimmerlicht reizabhängig. Die Durchschnittsfrequenz dieser Entladungen erwies sich nur wenig vom verwendeten Lichtreiz beeinflussbar. Elektroenzephalographische Weckreaktionen traten sowohl im Dunkeln, als auch im Licht, mit erhöhter Durchschnittsfrequenz der Zellenpotentiale gleichzeitig auf. Bei entkoppeltem Auge wurde während der Weckreaktion eine Erniedrigung der durchschnittlichen Entladungsfrequenz beobachtet. Aus diesen Befunden wird geschlossen, dass der laterale Knick auch von anderen Gebieten aus, nicht nur von der Netzhaut, beeinflusst wird.

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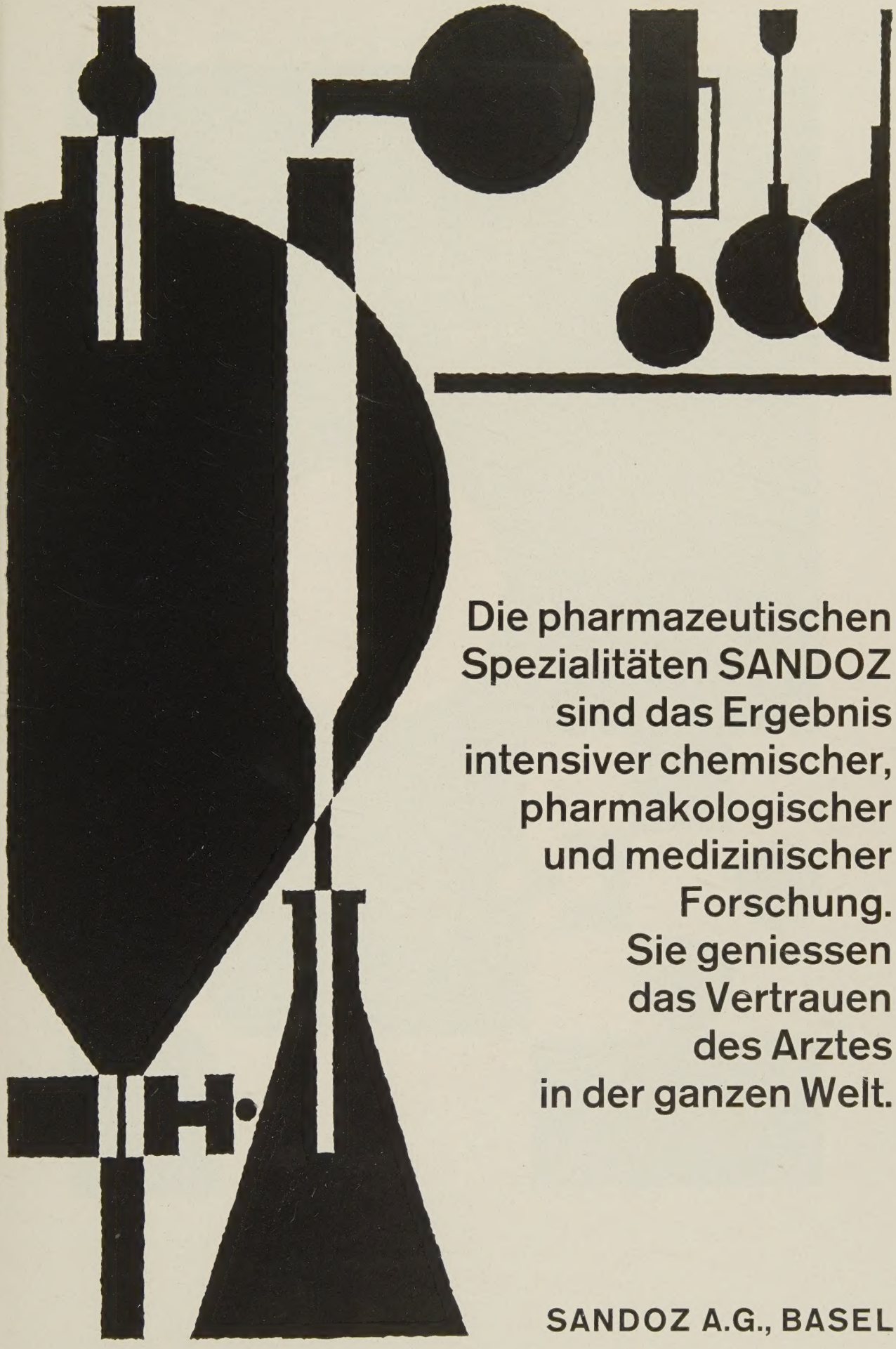
[10] S. Ramon y Cajal, *Histologie du système nerveux*, II (Madrid 1955), p. 392.



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